Prognostic and predictive markers for breast cancer management

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I am one of the PIs of the MINDACT study (but I have no financial interests related to MammaPrint).
PATIENT SELECTION (individualized treatment): WHY?

Outcomes of Adjuvant Chemotherapy in Breast Cancer

All patients with the same diagnosis

- No Benefit No Toxicity
- No Benefit + Toxicity
- + Benefit + Toxicity
- + Benefit No Toxicity

Walgren et al. JCO 2005;23:7342-7349
PATIENT SELECTION (individualized treatment): WHY?

Successive generations of adjuvant CT regimens

++ ADJUVANT AIs ++

+++ ADJUVANT TRASTUZUMAB+++
PATIENT SELECTION (individualized treatment): HOW?

2 MAIN QUESTIONS TO BE ANSWERED

WHO NEEDS TREATMENT?

WHICH TREATMENT IS BEST?

TREATMENT CHOICES

AVOID UNDER AND OVER TREATMENT

INDIVIDUALIZE TREATMENT

New/better PROGNOSTIC FACTORS

New/better PREDICTIVE FACTORS
# CLINICAL IMPLEMENTATION OF BIOMARKERS

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Analytical Validation</th>
<th>Clinical Validation</th>
<th>Clinical Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ANALYTICAL VALIDATION</strong></td>
<td>• Accuracy and prediction in measurement of the analytes</td>
<td>• Correlation of score/classifier with clinical state or outcome</td>
<td>• Actionable (could affect treatment)</td>
</tr>
<tr>
<td></td>
<td>• Robustness</td>
<td></td>
<td>• Use results for patient benefit</td>
</tr>
</tbody>
</table>
PROGNOSTIC AND/OR PREDICTIVE FACTORS

Prognostic

Predictive

Prognostic and predictive

Factor present

Factor absent

Adapted M. Buyse
Sources of variation in biomarkers testing

- Time to slicing and fixation
- Method of tissue processing
- Type of fixation
- Equipment calibration
- Laboratory procedures
- Assay validation
- Type of antigen retrieval
- Staff competence
- Test reagents
- Control materials
- Assay conditions
- Use of image analysis
- Interpretation criteria
- Reporting elements
- Scoring system
- Assay validation
- Staff competence
- Type of antigen retrieval
- Reporting elements

Wolff et al 2007

IHC, ISH testing variables

Post-analytical

Analytical
PROGNOSTIC FACTORS
CLINICOPATHOLOGICAL PROGNOSTIC FACTORS IN EBC

- Tumor size
- Lymph node status
- Grade
- ER, PR and HER-2 receptor expression
- Presence of lymphovascular invasion
PROGNOSTIC ALGORITHMS FOR TREATMENT DECISION MAKING

- Predict Plus
- Adjuvant! Online
- Nottingham Prognostic Index

INTERNATIONAL TREATMENT GUIDELINES

- ESMO, St. Gallen, NCCN, ASCO, AGO, Cancer Care Ontario Clinical Practice Guidelines, and others...
PROGNOSTIC VALUE OF BC MOLECULAR SUBTYPES

Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer

Alex Preit, Joel S. Parker, Olga Kharchenko, Cheng Pan, Chad Livery, Jason I. Henschke, Xiaping Hu, Charles M. Perou.

A

Relapse Free S

0.6

0.4

0.2

0.0

0

20

40

60

80

100

120

140

Months

p=7.67e-06

Basal-like
Claudin-low
HER2-enriched
Luminal A
Luminal B

CLINICAL IMPLICATIONS

This study provides insights into the molecular subtypes of breast cancer, which can aid in personalized treatment strategies.
## MOLECULAR CLASSIFICATION OF BREAST CANCER - SURROGATES

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Molecular characteristics</th>
<th>Histological characteristics SURROGATES</th>
<th>Biology/treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Luminal A</strong></td>
<td>• luminal CK expression</td>
<td>• ER+</td>
<td>• indolent behaviour</td>
</tr>
<tr>
<td></td>
<td>• resembles normal epithelium cells</td>
<td>• low grade/low proliferation</td>
<td>• sensitive to hormonal therapy</td>
</tr>
<tr>
<td><strong>Luminal B</strong></td>
<td>• <em>similar than luminal A</em></td>
<td>• ER+ (lower expression than in luminal A)</td>
<td>• more aggressive behaviour</td>
</tr>
<tr>
<td></td>
<td>• high grade/high proliferation</td>
<td>• high grade/high proliferation</td>
<td>• less sensitive to hormonal therapy than luminal A</td>
</tr>
<tr>
<td><strong>Basal-like</strong></td>
<td>• without expression of ER, PR and HER-2 genes</td>
<td>•“ Triple negative” (ER-, PR -, HER 2-)</td>
<td>• aggressive behaviour</td>
</tr>
<tr>
<td></td>
<td>• basal CK expression (CK5)</td>
<td>• high grade/high proliferation</td>
<td>• sensitive to chemotherapy</td>
</tr>
<tr>
<td></td>
<td>• expression of growth factors (EGFR, c-kit, HGF, IGF)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• BRCA disfunction</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• genetic instability</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Her-2 enriched</strong></td>
<td>• amplification of HER-2 gene and overexpression of HER-2 receptor</td>
<td>• HER 2+</td>
<td>• aggressive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• sensitive to anti-HER-2 therapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• sensitive to chemotherapy</td>
</tr>
</tbody>
</table>
PROGNOSTIC VALUE OF SUBTYPES IHC SURROGATES

IHC TRANSLATION OF MOLECULAR CLASSIFICATION

CRUCIAL ROLE OF HIGH QUALITY PATHOLOGY (and also cost-effective!)

<table>
<thead>
<tr>
<th>Variable</th>
<th>MammaPrint</th>
<th>Oncotype DX</th>
<th>Breast Cancer Index</th>
<th>PAM 50 ROR</th>
<th>EndoPredict Clin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provider</td>
<td>Agendia</td>
<td>Genomic Health</td>
<td>Biotheranostics</td>
<td>NanoString</td>
<td>Sividon</td>
</tr>
<tr>
<td>Type of assay</td>
<td>70-gene assay</td>
<td>21-gene recurrence score</td>
<td>2-gene ratio (H/I) and molecular grade index</td>
<td>50-gene assay</td>
<td>12-gene assay</td>
</tr>
<tr>
<td></td>
<td>Centralized</td>
<td>Centralized</td>
<td>Decentralized</td>
<td>Decentralized</td>
<td>Decentralized</td>
</tr>
<tr>
<td>Type of tissue sample</td>
<td>Fresh frozen or FFPE</td>
<td>FFPE</td>
<td>FFPE</td>
<td>FFPE</td>
<td>FFPE</td>
</tr>
<tr>
<td>Technique</td>
<td>DNA microarray, also qRT-PCR</td>
<td>qRT-PCR</td>
<td>qRT-PCR</td>
<td>qRT-PCR</td>
<td>qRT-PCR</td>
</tr>
<tr>
<td>Clinical application</td>
<td>Prognosis of N 0-3, ER+/ER neg, untreated Early Relapses</td>
<td>Prognosis in ER+/HER2 neg, N0-3, treated with TAM Early Relapses</td>
<td>Prognostic in ER+, prediction of response to TAM Early and Late Relapses</td>
<td>Originally for intrinsic subtyping, prognosis Early and Late Relapses</td>
<td>Prognosis for ER/HER-2 neg, N0 Early and Late relapses</td>
</tr>
<tr>
<td>Results presentation</td>
<td>Dichotomous, good or poor prognosis</td>
<td>Low, intermediate and high risk groups</td>
<td>Continuous variable</td>
<td>Continuous variable</td>
<td>Dichotomous, low or high risk</td>
</tr>
<tr>
<td>Level of evidence</td>
<td>I A for prognosis and CT decision</td>
<td>I A for prognosis and CT decision</td>
<td>I B for prognosis</td>
<td>I B for prognosis</td>
<td>I B for prognosis</td>
</tr>
<tr>
<td>FDA clearance</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>NO</td>
</tr>
</tbody>
</table>
FIRST GENERATION SIGNATURES
Recurrence Score/Oncotype DX®

- A RT-PCR-based gene signature that measures the expression of 21 genes (16 cancer-related genes and 5 reference genes)
- *It uses the Recurrence score (RS)* to predict the risk of distant relapse within 10 years
- Developed in **ER+**, under tamoxifen treatment
- Extensive retrospective validation; ongoing prospective validation

$p < .001$

*Paik, NEJM 2004*
Oncotype DX® in Node Negative BC
Paik, JCO 2006

- **NSABP B-20**: ER+, N0, CT (CMF regimen); 651 pts (227 TAM /424 TAM+CT)
- **High RS (≥ 31): benefited from CT** (RR 0.26 (95% CI, 0.13 -0.53), relative risk reduction in 10 yrs **27.6%** (SE 8.0%))
- **Low RS (< 18) no significant benefit from CT** (RR 1.31 (95% CI, 0.46 – 3.78), relative risk reduction in 10 yrs **-1.1%** (SE 2.2%))
Oncotype DX® in node-positive BC
Albain - Lancet Oncol 2010

- **SWOG 8814 trial** – postmenopausal women, ER+, N+, CT (CAF)
- 367 pts (TAM 148/ CAF-TAM 219)
- **No benefit of CT for pts with RS < 18** (p=0.97, HR 1.02 (95%: CI, 0.54–1.93))
- **Better DFS with CT for pts with high RS (≥31)** (p=0.033, HR 0.59 (CI95%: 0.35–1.01))
Oncotype DX® and response to anthracyclines
Gianni et al. JCO 2005

• The Recurrence Score (RS) was positively associated with the likelihood of pathologic complete response (pCR; measurement of gene expression, p=0.005)

• No predictive value to differentiate between different chemotherapy agents/regimens
TAILORx Methods: Treatment Assignment & Randomization
Accrued between April 2006 – October 2010

Preregister – Oncotype DX RS
(N=11,232) → Register (N=10,273)

- ARM A: Low RS 0-10
  (N=1629 evaluable)
  ASSIGN Endocrine Therapy (ET)

- Mid-Range RS 11-25
  (N=6711 evaluable)
  RANDOMIZE
  Stratification Factors: Menopausal Status, Planned Chemotherapy, Planned Radiation, and RS 11-15, 16-20, 21-25

- ARM B: Experimental Arm
  (N=3399)
  ET Alone

- ARM C: Standard Arm
  (N=3312)
  Chemo and ET

- ARM D: High RS 26-100
  (N=1389 evaluable)
  ASSIGN Chemo and ET

Joseph A. Sparano, MD
Results LOW RISK ARM (ET alone)

No. of events: 88 iDFS events and 30 deaths within 5 years of registration, including 18 recurrences (10 distant as first event), 15 second primary breast cancers, 43 other second primary cancers, 12 deaths without another event.
TAILORx Results - ITT Population: RS 11-25 (Arms B & C)

836 IDFS events (after median of 7.5 years), including 338 (40.3%) with recurrence as first event, of which 119 (23.8%) were distant.

**Primary Endpoint**
Invasive Disease-Free Survival

- Hazard Ratio Arm B vs. Arm C (95% CI)
  - Arm C: CHEMO + ET
  - Arm B: ET Alone
  - P = 0.26
  - 1.08 (0.94, 1.24)

**Secondary Endpoint**
Distant Recurrence-Free Interval

- Hazard Ratio Arm B vs. Arm C (95% CI)
  - Arm C: CHEMO + ET
  - Arm B: ET Alone
  - P = 0.48
  - 1.16 (0.85, 1.51)

**Other Secondary Endpoints**
Relapse-Free Interval

- Hazard Ratio Arm B vs. Arm C (95% CI)
  - Arm C: CHEMO + ET
  - Arm B: ET Alone
  - P = 0.33
  - 1.11 (0.96, 1.37)

Overall Survival

- Hazard Ratio Arm B vs. Arm C (95% CI)
  - Arm C: CHEMO + ET
  - Arm B: ET Alone
  - P = 0.89
  - 0.99 (0.79, 1.22)
TAILORx Results - ITT Population: All Arms (A, B, C & D)

9-Year Event Rates

- **RS 0-10 (Arm A)**
  - 3% distant recurrence with ET alone

- **RS 11-25 (Arms B & C)**
  - 5% distant recurrence rate overall
  - \( \leq 1\% \) difference for all endpoints
    - IDFS (83.3 vs. 84.3%)
    - DRFI (94.5 vs. 95.0%)
    - RFI (92.2 vs. 92.9%)
    - OS (93.9 vs. 93.8%)

- **RS 26-100 (Arm D)**
  - 13% distant recurrence despite chemo + ET
Prospective WSG Phase III PlanB trial:
Adjuvant 4xEC→4xDoc vs. 6xDocetaxel/Cyclophosphamide in high clinical and intermediate/high genomic risk ER+/HER2-neg EBC

- Age ≤75 years
- cM0
- free margins
- pN+
- pN0 high risk

- pT>2
- G2-3
- uPA/PAI-1↑
- HR-
- age <35 years

** HR-**

Randomization

Recurrence Score: after early amendment

0-3 LN and RS>11
or ≥ 4 LN

** Doc\textsubscript{75}C\textsubscript{600} x 6\textsuperscript{*} **

** E\textsubscript{90}C\textsubscript{600}x4 \rightarrow Doc\textsubscript{100} x4\textsuperscript{*} **

** Endocrine therapy\textsuperscript{*} **

* Endocrine Therapy and RT according to national guidelines

E: Epirubicin; Doc: Docetaxel; C: Cyclophosphamide

Presented by: Nadia Harbeck, MD
PlanB: Endpoints

Primary endpoint
Disease-free survival (DFS) for anthracycline-free regimen vs. standard chemotherapy in HER2-negative primary breast cancer.
- DFS = time from randomization to any relapse, secondary malignancy or death without recurrence
- Results expected by 2017

Secondary Endpoints
- Safety
- Overall survival

Extensive translational program
- Prospective evaluation of prognostic impact of Recurrence Score (RS) at a median follow up of 3 and 5 years
- Outcome in RS low-risk patients treated by endocrine therapy alone
- Prospective evaluation of the prognostic impact of an independent central pathological review vs RS

Courtesy of Nadia Harbeck
PlanB: Translational subprotocol
5-year DFS in per-protocol population
(no chemotherapy in pN0-1 and Recurrence Score 0-11)

Gluz et al, EBCC 2016, plenary lecture

Presented by: Nadia Harbeck, MD

Courtesy of Nadia Harbeck
PlanB: Shared decision making based on Recurrence Score

- 18% of patients potentially spared chemotherapy (n=404 post-amendment) → 86% acceptance

Courtesy of Nadia Harbeck
PlanB: Recurrence Score by (central) Ki-67

RS result:
- >30
- 26-30
- 19-25
- 12-18
- 0-11

Courtesy of Nadia Harbeck
Gluz et al. JCO 2016
Development of 70 gene expression profile (MammaPrint®)

Tumor samples of known clinical outcome

Unbiased full genome gene expression analysis

Prognosis reporter genes

Distant metastases group

No distant metastases group

~4% die of breast cancer
~96% survive breast cancer

~50% die of breast cancer
~50% survive breast cancer

Courtesy & adapted from L van ’t Veer

INDEPENDENT VALIDATION: DESIGN

Boye et al, JNCI 98: 1183-1192, 2006

INDEPENDENT VALIDATION: DESIGN

- Target n = 400
  - RNA
  - Achieved n = 307

Amsterdam
- Gene expression profiling
  - Agilent platform
  - 70-gene prognostic custom designed chip

High or low gene signature risk

Clinical data
- «Local» pathological data

Audited clinical data
- Centrally reviewed path data (Milan)

Brussels
- Comparison of clinical vs gene signature assessment of prognostic risk

Endpoints
1. TDM
2. OS
3. DMFS, DFS

OVERALL SURVIVAL by GENE SIGNATURE RISK
Amsterdam/Agendia Signature

10-year OS 89% (81%-94%)

10-year OS 70% (62%-76%)

Average Survival HR ≈ 2.66

ADJUVANT! ONLINE FOR BREAST CANCER
Updated version

“Clinical low risk” defined as predicted 10-year BC survival probability
- ≥ 88% for ER+ patients
- ≥ 92% for ER- patients
MAMMAPRINT® (70-gene profile) in LN 1 to 3+ BC

Distant metastases as first event

- Good profile (n=142)
  - 91%
  - HR 3.8
    - (2.0 – 7.4; p<0.001)
    - HR adjusted 2.8
      - (1.3 – 6.0; p=0.009)

- Poor profile (n=205)
  - 73%
  - HR 6.1
    - (2.8 – 13.5; p<0.001)
    - HR adjusted 5.4
      - (2.1 – 13.9; p=0.001)

Breast cancer specific survival

- Good profile (n=142)
  - 96%
  - HR 3.8
    - (2.0 – 7.4; p<0.001)

- Poor profile (n=205)
  - 71%
  - HR 6.1
    - (2.8 – 13.5; p<0.001)
Chemotherapy benefit in **MammaPrint HIGH RISK** patients (n=289)

Knauer et al., Breast Cancer Res Treat, 2010 Feb
10 year follow-up of the RASTER study (2004-2006)

- Prospective evaluation of the MammaPrint® in community based hospitals.
- 427 breast cancer patients of 60 years or younger with cT1-3N0M0.
- Decision on adjuvant systemic treatment was based on:
  - Dutch guideline (CBO 2004)
  - Preference of patient and physician
  - High or Low Genomic risk of distant recurrence (MammaPrint)

MP Low-risk, no chemotherapy (n=185): 10 years DRFI 93.6%

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Patients</th>
<th>Received chemotherapy (%)</th>
<th>5 years DRFI (%)</th>
<th>10 years DRFI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MammaPrint Low-risk</td>
<td>219</td>
<td>34 (15.5)</td>
<td>96.3</td>
<td>93.7</td>
</tr>
<tr>
<td>MammaPrint High-risk</td>
<td>208</td>
<td>168 (80.8)</td>
<td>92.2</td>
<td>86.8</td>
</tr>
<tr>
<td>Clinical low-risk*</td>
<td>243</td>
<td>44 (18.1)</td>
<td>97.1</td>
<td>91.7</td>
</tr>
<tr>
<td>Clinical high-risk*</td>
<td>183</td>
<td>157 (85.8)</td>
<td>90.6</td>
<td>88.2</td>
</tr>
</tbody>
</table>

Log-rank P = 0.034

S. Vliek, ESMO 2017

*according to MINDACT
**MINDACT TRIAL DESIGN**

**Registration & Screening**
- Surgery

**N = 6600**

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

**Genomic (G) risk**
- (70-gene signature)

- **C-high/ G-high**
  - Discordant cases
  - C-high/G-low or C-low/G-high
  - 1st randomization to treatment
    - use Clinical vs. Genomic risk
    - 2nd randomization
      - Anthracycline -based vs. Capecitabine-Docetaxel
    - Endocrine therapy
    - HR+
  - 3rd randomization
    - Tamoxifen 2y / Letrozole 5y vs. Letrozole 7y

- **C-low/G-low**
  - No Chemotherapy
  - HR+

**Registration & Screening**
- Surgery
Primary endpoint: Distant metastasis free survival (DMFS) at 5 years
Null hypothesis: 5-year DMFS rate in PT population = 92%
Alpha: 2.5% (1-sided)
Power: 80% when true 5-year DMFS rate=95%

Primary test:
95% 2-sided Confidence interval (CI) for the 5-year DMFS rate will be compared to 92%
significant if CI exceeds 92%

F. Cardoso, NEJM 2016
MINDACT population at 5y median follow-up
DMFS IN ALL 4 RISK GROUPS

Distant Metastasis Free Survival

% at 5 year
- cL/gL: 97.6 (96.9, 98.1)
- cL/gH: 94.8 (92.4, 96.4)
- cH/gL: 95.1 (93.8, 96.2)
- cH/gH: 90.6 (89.0, 92.0)

Discordant risk groups

F. Cardoso, NEJM 2016
The primary statistical test (DMFS at 5Y)

### Null Hypothesis: set at 92%

- Observed 5Y DMFS = 94.7%
- 95% CI ≈ 92.5 – 96.2% excludes 92% !!!

F. Cardoso, NEJM 2016
Efficacy Secondary Endpoint: CT vs no CT in discordant risk groups in ITT analysis

Distant Metastasis Free Survival
c-High/g-Low

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% at 5 Year(s) (95% CI)</th>
<th>Hazard Ratio (adjusted Cox model) (95% CI)</th>
<th>p-value (adjusted logrank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>95.9 (94.0, 97.2)</td>
<td>0.78</td>
<td>0.267</td>
</tr>
<tr>
<td>no CT</td>
<td>94.4 (92.3, 95.9)</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

Distant Metastasis Free Survival
c-Low/g-High

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% at 5 Year(s) (95% CI)</th>
<th>Hazard Ratio (adjusted Cox model) (95% CI)</th>
<th>p-value (adjusted logrank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>95.8 (92.9, 97.6)</td>
<td>1.17</td>
<td>0.657</td>
</tr>
<tr>
<td>no CT</td>
<td>95.0 (91.8, 97.0)</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

F. Cardoso, NEJM 2016
The MINDACT population: CT assignment according to a “Clinical” vs a “Genomic” strategy

Whole population N = 6,693

- Discordant N=2745 clinical Low/genomic Low
- N=1806 clinical High/genomic High
- N=1550 clinical High/genomic Low
- N=592 clinical Low/genomic High

«Clinical» strategy CT to 1550 + 1806 = 3,356 pts = 50 %

«Genomic» strategy CT to 592 + 1806 = 2,398 pts = 36 %

14% reduction

F. Cardoso, NEJM 2016
Proposed future clinical use of MammaPrint®

Clinical risk (c)
Adjuvant Online!

Genomic risk (g)
70-gene signature or MammaPrint®

c-Low/g-Low
Discordant

c-Low/g-High  c-High/g-Low

R-T
N=1550  N=1806

Clinical «Low risk» patients

Clinical «High risk» patients

No proven added value of MammaPrint®

Proven added value of MammaPrint® with a 46%\([1550/(1550+1806)]\) reduction in CT prescription (depends on baseline CT prescription rate!)

F. Cardoso, NEJM 2016
Conclusions (2)

• Mindact results provide level 1A evidence of the clinical utility of MammaPrint® for assessing the lack of a clinically relevant chemotherapy benefit in the clinically high risk (c-High) population.

• c-High/g-Low patients, including 48% Node positive and 29% grade 3, had a 5-year DMFS rate in excess of 94%, whether randomized to adjuvant CT or no CT.

• In the entire MINDACT population, the trial confirmed the hypothesis that the « genomic » strategy leads to a 14% reduction in CT prescription versus the « clinical » strategy.

• Among the c-High risk patients, the clinical use of MammaPrint® is associated with a 46% reduction in chemotherapy prescription.

F. Cardoso, NEJM 2016
Practical use of Mammaprint® in the clinic based on evidence from the MINDACT trial

HR+ tumor: Define clinical risk

Clinical “low” risk*
- Treatment according to guidelines

Clinical “high” risk
- Discuss with patient if she would value a 1.5% gain in DMFS with adjuvant chemotherapy
  - No
    - Order Mammaprint
  - Yes
    - Proceed with chemotherapy

*Courtesy M. Piccart
A GOLDMINE FOR (Future) RESEARCH

FROZEN TUMOR SAMPLES

PARAFFIN-EMBEDDED TUMOR SAMPLES

SERUM & BLOOD SAMPLES

Independent biological materials bank
Policy for access to samples and/or data
The use of MammaPrint allows to spare chemotherapy in about 46% of patients traditionally considered at high risk.

Efficacy: CT vs no CT in discordant risk groups Intent-to-treat analysis

Implementation problems:
Cost of the test, lack of reimbursement, logistics, time
Gene expression profiling predicts clinical outcome of breast cancer

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Breast cancer patients with the same stage of disease can have markedly different treatment responses and overall outcome. The

- 14 years
- ± 50 million Euros
- Over 11,000 pts screened & 6,600 enrolled
First-generation Gene Signatures for EBC Recurrence Prediction

Time dependence of HRs for gene signature adjusted for the clinical risk (A) and the clinical risk alone (B) for time to distant metastases

Oncotype Dx RS, Mammaprint and Rotterdam gene signature are good in predicting early distant recurrence of EBC.

## DISSECTING GENE EXPRESSION SIGNATURES

<table>
<thead>
<tr>
<th>Microarray Indices</th>
<th>Amsterdam No (%)</th>
<th>Rotterdam No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>70 genes</td>
<td>76 genes</td>
</tr>
<tr>
<td>ESR1 = luminal/basal</td>
<td>35 (50)</td>
<td>17 (18)</td>
</tr>
<tr>
<td>ERBB2 = Her2-neu</td>
<td>6 (9)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>STK6 = proliferation/GGI</td>
<td>34 (49)</td>
<td>30 (39)</td>
</tr>
<tr>
<td>PLAU = stroma/invasion</td>
<td>10 (14)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>STAT1 = immune response</td>
<td>4 (6)</td>
<td>6 (8)</td>
</tr>
<tr>
<td>VEGF = angiogenesis</td>
<td>7 (10)</td>
<td>4 (5)</td>
</tr>
<tr>
<td>NA = undetermined</td>
<td>9 (9)</td>
<td>30 (39)</td>
</tr>
</tbody>
</table>
SECOND GENERATION SIGNATURES
PAM 50 ROR (PROSIGNA®)

- PROSIGNA ROR score is based on gene expression profile, proliferation score and tumor size
- 1017 pts in the ATAC (Dowsett, JCO 2013) and 1620 pts in the ABCSG8 (Gnant, Ann Oncol 2014)
- Developed in patients under ET therapy
- Adds prognostic information to standard clinicopathological parameters (within first 10 yrs of follow up)
- RNA-based multigene test
- Used to predict the likelihood of distant relapse in ER+ HER-2 negative EBC pts treated with adjuvant ET
- **EP clin**: combining the EP score, tumor size and nodal status
- Validated in the ABCSG-6 and ABCSG-8 trials;
  - identified a subgroup of pts with an excellent long-term prognosis after a standard 5 yrs of ET
EP-clinic-score

98.20% (96.5-99.8)

87.7% (82.8-92.5)

Allow the identification of pts with an excellent prognosis after 5 yrs, for whom it might not be necessary to extend endocrine therapy

Courtesy F. Penault-Llorca
Breast Cancer index (BCI)

- a second-generation gene signature that is prognostic for pts with ER+ EBC who have/have not received TAM

- a qRT-PCR method that measures expression of 2 genes, HOXB13 and IL17BR and classifies pts into low, intermediate and high-risk group

- Validation: the Stockholm study, n=317 pts, ER+, N0, TAM

Sgroi DC et al., Lancet Oncol 2013.

BCI was the only significant prognostic factor for risk of both, early and late distant recurrence (apart from OncotypeDx RS and IHC4, which predicted only early distant recurrence).

Sgroi DC et al., Lancet Oncol 2013.
DO GENOMIC TESTS REPLACE CLASSICAL PROGNOSTIC FACTORS OR ADD TO THEM?

Genomic
- poor-vs-good
- grade (2+3 vs 1)
- ER-negative
- node-positive
- size >2cm
- age >50y

Risk factors:
- Hazard ratio:
- P-value:
  - 3e-05 0.004
  - 1e-06 0.03
  - 0.05 0.9
  - 1e-07 0.2
  - 0.002 0.006
  - 0.5 0.5

Clinico-pathological

Untreated

Treated

C. Sotiriou et al
% of pts classified as low/intermediate risk:

82.1% for Oncotype DX
72.0% for IHC4
65.6% for Prosigna
61.4% for MammaPrint
• 774 post-menop, ER+ BC pts who received ET for 5 years and did not receive CT; Trans-ATAC cohort

• Primary objective: To compare the prognostic value of multigene signatures RS (RSPC), ROR, BCI, EPClin, IHC4, in addition to the CTS for distant recurrence for 0 to 10 years and 5 to 10 years after diagnosis
  • CTS (clinical treatment score): nodal status, tumor size, grade, age, and ET
  • IHC4: ER, PR, Ki67, and ERBB2
  • RS-pathology-clinical (RSPC) score (RS+ clinical characteristics): web tool
  • RS and BCI- only molecular
  • ROR (tumor size), EP Clin (tumor size, LN)

• Primary endpoint: time to distant recurrence
- **N0**: All signatures provide independent prognostic information; **ROR, EPClin, BCI** more potent
- **N1-3**: weaker prognostic significance, and not significant for IHC4
- **N0**: **EP Clin, BCI and ROR** are better for late relapses but identify larger proportion of high risk at 10 years
- **Combination of clinical and molecular information** enhanced prognostic performance.

### Table 1. Univariate HRs and C Indexes for All Prognostic Signatures According to Nodal Status During Years 0 to 10

<table>
<thead>
<tr>
<th>Gene Signature</th>
<th>Node-Negative Disease (n = 591)</th>
<th>Node-Positive Disease (n = 227)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)²</td>
<td>C Index (95% CI)</td>
</tr>
<tr>
<td>CTS</td>
<td>1.99 (1.58-2.50)</td>
<td>0.721 (0.668-0.774)</td>
</tr>
<tr>
<td>IHC4</td>
<td>1.95 (1.55-2.45)</td>
<td>0.725 (0.665-0.785)</td>
</tr>
<tr>
<td>RS</td>
<td>1.69 (1.40-2.03)</td>
<td>0.667 (0.585-0.750)</td>
</tr>
<tr>
<td>BCI</td>
<td>2.46 (1.88-3.23)</td>
<td>0.762 (0.704-0.820)</td>
</tr>
<tr>
<td>ROR</td>
<td>2.56 (1.96-3.35)</td>
<td>0.764 (0.707-0.821)</td>
</tr>
<tr>
<td>EPclin</td>
<td>2.14 (1.71-2.68)</td>
<td>0.765 (0.716-0.814)</td>
</tr>
</tbody>
</table>
COST-EFFECTIVENESS ANALYSIS OUTCOME:

Incremental Cost–Effectiveness Ratio = \( \frac{\text{cost of treatment} - \text{cost of comparator}}{\text{Effect(iveness) of treatment} - \text{effect(iveness) of comparator}} \)

“Effectiveness” is expressed in QALYs

QALY is Quality adjusted life year

New technology is accepted if ICER is below the maximum willingness to pay per QALY (e.g. €30,000/QALY)
## COST-EFFECTIVENESS OF GENOMIC TESTING > PUBLISHED STUDIES

<table>
<thead>
<tr>
<th></th>
<th>N=34 Cost-effectiveness analyses</th>
<th>N=32 Genomic profile resulted as preferred strategy</th>
<th>ICER ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncotype DX</td>
<td>26</td>
<td>25</td>
<td>Dominance- €39,000/QALY</td>
</tr>
<tr>
<td>MammaPrint</td>
<td>7</td>
<td>6</td>
<td>Dominance- €134,000/QALY</td>
</tr>
</tbody>
</table>
### COST-EFFECTIVENESS BASED ON MINDACT RESULTS

**RESULTS: BUDGET IMPACT**

<table>
<thead>
<tr>
<th></th>
<th>Breast cancer incidence</th>
<th>Per patient savings</th>
<th>Annual savings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UK</strong></td>
<td>~20,000</td>
<td>£1,447</td>
<td>£28M</td>
</tr>
<tr>
<td><strong>NL</strong></td>
<td>~4,000</td>
<td>€9,215</td>
<td>€37M</td>
</tr>
<tr>
<td><strong>US</strong></td>
<td>~120,000</td>
<td>$12,625</td>
<td>$1,5B</td>
</tr>
</tbody>
</table>

*Courtesy V. Retèl*
BIOMARKER
Ready for use routine use in the clinic?

TECHNICAL VALIDATION
The test is
* sensitive
* specific
* reproducible

CLINICAL VALIDATION
The test identifies subsets with significantly different
* risks of relapse
* chances of response

YES
YES (Different LoE)

GENOMIC TESTS
GENOMIC TESTS IN ALL OR ONLY SELECTED BREAST CANCER CASES?

**TRIPLE NEGATIVE**
(ER-, PR-, HER-2 neg)
- CT indispensible

**LUMINAL**
ER+ HER-2 neg
- “Clear” indication from classical factors
- All LOW risk: high levels ER, PR, grade 1, node negative, low proliferation
- All HIGH risk: low levels ER, PR, grade 3, node positive, high proliferation
- CT → HT

**HER-2 POSITIVE**
- CT + anti-HER indispensible

**CRUCIAL IMPORTANCE OF HIGH QUALITY PATHOLOGY**
- HT alone
- “No Clear” indication from classical factors; some high & some low risk

GENOMIC TEST
EARLY BREAST CANCER: WHO NEEDS ADJUVANT CT?

- CLINICAL/PATHOLOGICAL/GENOMIC FACTORS ARE BEST USED IN COMBINATION.
- Responsiveness is a continuum.
- PATIENT PREFERENCE!

Abbreviations: ER, estrogen receptor; GGI, genomic grade index.

PREDICTIVE FACTORS
What is the level of prediction accuracy clinically useful?

Breast Cancer

HER2

Negative predictive value

HIGH 95%

(<5% chance to respond to anti-estrogens or trastuzumab)

ER/PGR

Positive predictive value

30-50%

Cut off 1%

Courtesy F. Penault-Llorca
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Prognostic</th>
<th>Predictive</th>
<th>Technical validation</th>
<th>Clinical validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>++</td>
<td>+++</td>
<td>YES LOE Ib</td>
<td>YES</td>
</tr>
<tr>
<td>PgR</td>
<td>+++</td>
<td>+</td>
<td>YES LOE Ib</td>
<td>NO</td>
</tr>
<tr>
<td>HER2</td>
<td>++</td>
<td>+++</td>
<td>YES LOE Ib</td>
<td>YES</td>
</tr>
<tr>
<td>Ki67</td>
<td>++</td>
<td>+</td>
<td>NO</td>
<td>NO</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Test and scoring recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>IHC</td>
</tr>
<tr>
<td>PgR</td>
<td>IHC</td>
</tr>
<tr>
<td>HER2</td>
<td>IHC ≥10% cells with complete membrane staining ISH: number of HER2 gene copies ≥6 or the ratio HER2/chromosome 17 ≥ 2</td>
</tr>
<tr>
<td>Ki67</td>
<td>IHC no final consensus on cut-off around 20% (Ki67 &lt; 10% = low; Ki67 &gt; 30% = high)</td>
</tr>
</tbody>
</table>

Courtesy F. Penault-Llorca
Prediction of response to NACT

Histologic subtype (lobular vs ductal)
High Tumor grade
ER negative
HER2 positive
SBR grade, proliferation
Intrinsic classification
High TILs

Courtesy F. Penault-Llorca
Association between pCR and EFS by BC subtype

PROGNOSTIC VALUE OF pCR

Overall survival as a function of response to neoadjuvant PCT

Liedtke C et al, J Clin Oncol, 2008, 26:1275
The magnitude of improvement in pCR rate did not predict EFS and OS effect

PREDICTIVE FACTORS
Endocrine-based strategies
Many genetic biomarkers have shown little or no association with response to therapy

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Clinical studies</th>
<th>Findings (mutant/amplified/loss vs wildtype)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3CA</td>
<td>BOLERO-2, PALOMA-3</td>
<td>PIK3CA: no significant difference in treatment effect</td>
</tr>
<tr>
<td>CCND1</td>
<td>BOLERO-2, PALOMA-1</td>
<td>CCND1 (BOLERO-2): no significant difference in treatment effect</td>
</tr>
<tr>
<td>p16</td>
<td>PALOMA-1</td>
<td>CCND1/p16 (PALOMA-1): changes in copy number did not improve patient selection beyond ER/HER2 status</td>
</tr>
<tr>
<td>FGFR</td>
<td>BOLERO-2</td>
<td>FGFR: no significant difference in treatment effect</td>
</tr>
</tbody>
</table>

**ESR1 mutations seem to be associated with resistance to AIs**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Clinical studies</th>
<th>Findings (mutant/amplified/loss vs wildtype)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR1</td>
<td>PALOMA-3</td>
<td>PALOMA-3: no difference between mut vs wt</td>
</tr>
<tr>
<td></td>
<td>SoFEA</td>
<td>SoFEA: treatment less effective in mut vs wt</td>
</tr>
<tr>
<td></td>
<td>BOLERO-2</td>
<td>BOLERO-2: improved OS and PFS in wt vs mut*</td>
</tr>
<tr>
<td></td>
<td>Schiavon et al</td>
<td>Schiavon et al: ESR1 mutations predict resistance to subsequent AI therapy**</td>
</tr>
</tbody>
</table>

*no statistical analysis carried out; **small sample size (n=45); mut = mutant; wt = wildtype

ESR1 mutation and selection of endocrine therapy

ESR1-Mutations result in constitutively activated ER leading to resistance -> SERD more sensitive than AI or SERM

Incidences of ESR1-Mutations

- Primary: 1% ESR1-Mutations, 99% ESR1-WT
- Early MBC: 8% ESR1-Mutations, 92% ESR1-WT
- Late MBC: 20% ESR1-Mutations, 80% ESR1-WT

ESR1-WT: No difference between Exemestane and Fulvestrant

ESR1-Mutant tumours: less sensitive to Exemestane

ESR1-status makes no difference in response to SERDs


ESR1-Mutations result in constitutively activated ER leading to resistance -> SERD more sensitive than AI or SERM


 Courtesy Peter Schmid, ESMO 2016, Discussant
Potential Mechanisms of Acquired Resistance to CDK4/6 Inhibitors

- RB loss*
- Cyclin E1 over-expression*
- Lineage plasticity** (via epigenetics)

sensitivity to CDK4/6 inhibitors may potentially be retained


The RBsig Predicts Resistance to Palbociclib

In Vitro and Clinical Outcome

“In vitro”

METABRIC dataset

Luminal A, endocrine treated
HR = 2.67 (1.8-3.9, P = 1.1e-06)

Luminal B, endocrine treated
HR = 2.31 (1.3-4.1, P = 0.0017)

AUC: 0.7778

Adjuvant CT in ER+ EARLY BREAST CANCER?
All patients with sufficient high risk!

• How to accurately evaluate risk?
  • How to define high risk?

• No predictive biomarker for specific CT agents
• General “predictive” markers for CT (highly proliferative tumors, whichever way you measure proliferation...)

In Conclusion:

ER+ → Adjuvant ET
HER2+ → Adjuvant anti-HER2 therapy