PROGNOSTIC AND PREDICTIVE MARKERS IN BREAST CANCER

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EORTC Breast Group Past-Chair
DISCLOSURES

Consultant/Ad Board:

Amgen, Astellas/Medivation, AstraZeneca, Celgene, Daiichi-Sankyo, Eisai, GE Oncology, Genentech, GlaxoSmithKline, Macrogenics, Merck-Sharp, Merus BV, Mylan, Mundipharma, Novartis, Pfizer, Pierre-Fabre, Roche, Sanofi, Seattle Genetics, Teva

I am one of the PIs of the MINDACT study (but 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 + Toxicity
- + Benefit + Toxicity
- + Benefit No Toxicity

No Benefit No Toxicity

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

Successive generations of adjuvant CT regimens

- +++ ADJUVANT TRASTUZUMAB+++  
- ++ ADJUVANT AIs ++

Financial toxicity

- d) \( \approx \) 20,000 $  
- c) \( \approx \) 13,800 $  
- b) \( \approx \) 7,400 $  
- a) \( \approx \) 800 $  

Adapted from G. Hortobagyi
PATIENT SELECTION (individualized treatment): WHY?

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
PROGNOSTIC AND/OR PREDICTIVE FACTORS

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
- Staff competence
- Type of antigen retrieval
- Test reagents
- Control materials
- Assay conditions
- Use of image analysis
- Interpretation criteria
- Reporting elements
- Scoring system

IHC, ISH testing variables

Pre-analytical

Post-analytical

Analytical

Wolff et al 2007
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
• Nottingham Prognostic Index
• Adjuvant! Online

• INTERNATIONAL TREATMENT GUIDELINES
  ▪ St. Gallen Guidelines, NCCN, ASCO, ESMO, 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

A

Luminal
HER2
Basal
Immune
Cell Adhesion
Mesenchymal/ECM
Proliferation

+2 +1 0 -1 -2

Claudin-low (CL)
Normal Breast-like (NBL)
Basal-like (BL)
Luminal A and B (LA and LB)
HER2-enriched (H2)

Relapse Free S

0.0 0.2 0.4 0.6

0 20 40 60 80 100 120 140

Courtesy of MJ Brito

p=7.67e-06

Courtesy of MJ Brito
PROGNOSTIC VALUE OF SUBTYPES IHC SURROGATES

IHC TRANSLATION OF MOLECULAR CLASSIFICATION

ER/PR  HER2  PCAD  CK5  EGFR  CK14

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


Courtesy of MJ

FIRST GENERATION GENOMIC 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

<table>
<thead>
<tr>
<th>Variable</th>
<th>P Value</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analysis without recurrence score</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at surgery</td>
<td>0.004</td>
<td>0.57 (0.39–0.83)</td>
</tr>
<tr>
<td>Clinical tumor size</td>
<td>0.06</td>
<td>1.44 (0.99–2.11)</td>
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<tr>
<td><strong>Analysis with recurrence score</strong></td>
<td></td>
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</tr>
<tr>
<td>Age at surgery</td>
<td>0.08</td>
<td>0.71 (0.48–1.05)</td>
</tr>
<tr>
<td>Clinical tumor size</td>
<td>0.23</td>
<td>1.26 (0.86–1.86)</td>
</tr>
<tr>
<td>Recurrence score</td>
<td>&lt;0.001</td>
<td>3.21 (2.23–4.61)</td>
</tr>
</tbody>
</table>

Paik, NEJM 2004
Recurrence Score/Oncotype DX®

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® 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 TRIAL

Enrollment period: April 7, 2006 to October 6, 2010 (N=10,273 eligible)

Key Eligibility Criteria
- Node-negative
- ER-pos, HER2-neg
- T1c-T2 (high-risk T1b)
- Age 18-75 years
- No PBI planned

Statistical Design
- RS 11-25: non-inferiority
  - 90% vs. <87% iDFS
  - 835 DFS events
- RS < 11
  - 95% vs. <93% DRFI at 10 years
  - 75 DRFI events

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

5 year iDFS Rate
93.8%
(95% CI 92.4%, 94.9%)

5 year DRFI Rate
99.3%
(95% CI 98.7%, 99.6%)

5 year RFI Rate
98.7%
(95% CI 97.9%, 99.2%)

5 year OS Rate
98.0%
(95% CI 97.0%, 98.6%)
PlanB: Design
HER2-negative early breast cancer

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

HR-

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

HR+

0-3 LN and RS > 11
or ≥ 4 LN

0-3 LN and RS ≤ 11

Recurrence Score: after early amendment

RANDOMIZATION

Doc_{75C600} x 6*

E_{90C600} x 4 \rightarrow Doc_{100} x 4*

Endocrine therapy*

* Endocrine Therapy and RT according to national guidelines
E: Epirubicin; Doc: Docetaxel;
C: Cyclophosphamide

Presented by: Nadia Harbeck, MD

Presented at: ASCO Annual Meeting '17 | #ASCO17

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

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

Dropout/ non-compliance rates

- RS>25: 10%
- RS 12-25: 21%
  - N0 patients 25%
  - N1 patients 15%
- RS 0-11: 1.5%; CT in 5.5% N0 and 25% N1 patients
**PlanB: Translational subprotocol**

5-year DFS in per-protocol population
(no chemotherapy in pN0-1 and Recurrence Score 0-11)

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Presented by: Nadia Harbeck, MD

Gluz et al, EBCC 2016, plenary lecture

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**Disease free survival**

- Recurrence Score Groups
  - 0-11
  - 12-25
  - >25

- 5-Y DFS 94.2%  
- 5-Y DFS 94.5%  
- 5-Y DFS 85.5%

- N0 5-Y DFS 94%  
- N1 5-Y DFS 88%

Gluz et al, EBCC 2016, plenary lecture

Presented by: Nadia Harbeck, MD
PlanB trial (HR+/HER2- population; 5-year median follow-up): Conclusions II

- Both local and central grade 3 are independent prognostic markers, despite their significant assessment discordance.
- Ki-67 correlates well with Recurrence Score in (central) Ki-67 ranges <10% and ≥40%.
- Highest prognostic utility from a multigene assay can be expected in cases with intermediate Ki-67.
- Only pN2-3, continuous RS, grade 3 and tumor size >2cm are independent predictors for DFS and should be used together for treatment decisions in early HR+ HER2-breast cancer.

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

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

0-9
n = 509

10-19
n = 910

20-29
n = 592

30-39
n = 160

>39
n = 99

0% 20% 40% 60% 80% 100%
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

ER+ and ER –
Untreated patients


Courtesy & adapted from L van ‘t Veer
INDEPENDENT VALIDATION : DESIGN

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

**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
- Genetic low risk: 89% (81%-94%)
- Genetic high risk: 70% (62%-76%)

Patient Information
- Age:
- Comorbidity: Average for Age
- ER Status: Positive
- Tumor Grade: Grade 3
- Tumor Size: 2-3 cm
- Positive Nodes: 0
- Calculate For: Mortality
- 10 Year Risk: 54

**ADJUVANT! ONLINE FOR BREAST CANCER**
Updated version

- No additional therapy:
  - 72.2 alive in 10 years
  - 25.6 die of cancer

“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+ BC

Distant metastases as first event

<table>
<thead>
<tr>
<th>Profile</th>
<th>Events</th>
<th>10-Year Event Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good profile</td>
<td>142</td>
<td>91%</td>
</tr>
<tr>
<td>Poor profile</td>
<td>205</td>
<td>73%</td>
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</table>

Breast cancer specific survival

<table>
<thead>
<tr>
<th>Profile</th>
<th>Events</th>
<th>10-Year Event Rate</th>
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<td>Good profile</td>
<td>142</td>
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<tr>
<td>Poor profile</td>
<td>205</td>
<td>71%</td>
</tr>
</tbody>
</table>

HR adjusted 5.4
(2.1 – 13.9; p=0.001)

HR adjusted 2.8
(1.3 – 6.0; p=0.009)
Chemotherapy benefit in **MammaPrint** HIGH RISK patients (n=289)

**Clinical Trial**

The predictive value of the 70-gene signature for adjuvant chemotherapy in early breast cancer

Michael Knauer · Stella Mook · Emiel J. Michael Hauptmann · Marc J. van de Vij Jolien M. Bueno-de-Mesquita · Sabine C.

Received: 27 January 2010 / Accepted: 19 February © Springer Science+Business Media, LLC. 2010

**DDFS: MammaPrint LOW RISK** (n=252)

- **ET+CT** (n=78, 31%)
- **ET** (n=174, 69%)

**HR.0.26(0.03-2.02)**

**p=0.20**

**99%**

**93%**

**PERCENT SURVIVAL**

**TIME IN YEARS**

**DDFS: MammaPrint HIGH RISK** (n=289)

- **ET+CT** (n=148, 51%)
- **ET** (n=141, 49%)

**HR.0.35(0.17-0.71)**

**p=0.01**

**88%**

**76%**

**PERCENT SURVIVAL**

**TIME IN YEARS**

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>
MINDACT TRIAL DESIGN

Registration & Screening Surgery

N= 6694

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

Chemotherapy

2nd randomization
Anthracycline-based vs. Capecitabine-Docetaxel

Endocrine therapy

3rd randomization
Tamoxifen 2y / Letrozole 5y vs. Letrozole 7y

No Chemotherapy

C-low/G-low

HR+
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)

F. Cardoso, NEJM 2016
MINDACT population at 5y median follow-up

DISCORDANT RISK GROUPS: PRIMARY TEST

The primary statistical test
(DMFS at 5Y)

Distant Metastasis Free Survival
cHgL no ACT

Null Hypothesis: set at 92%

Observed 5Y DMFS = 94.7%

95% CI ≈ 92.5 – 96.2% excludes 92% !!!

F. Cardoso, NEJM 2016
Efficacy: CT vs no CT in discordant risk groups
Intent-to-treat analysis

**Distant Metastasis Free Survival**

- **c-High/g-Low**
  - CT: 95.9% (94.0, 97.2) at 5 years, Hazard Ratio: 0.78 (0.50, 1.21), p-value: 0.267
  - no CT: 94.4% (92.3, 95.9)

- **c-Low/g-High**
  - CT: 95.8% (92.9, 97.6) at 5 years, Hazard Ratio: 1.17 (0.59, 2.28), p-value: 0.657
  - no CT: 95.0% (91.8, 97.0)

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

Whole population N = 6,693

- N=2745 clinical Low/genomic Low
- N=592 clinical Low/genomic High
- N=1550 clinical High/genomic Low
- N=1806 clinical High/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) | Genomic risk (g)
Adjuvant Online! | 70-gene signature or MammaPrint®

c-Low/g-Low | Discordant | c-High/g-High

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*
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.

## Microarray Indices

<table>
<thead>
<tr>
<th>Gene</th>
<th>Amsterdam No (%)</th>
<th>Rotterdam No (%)</th>
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</thead>
<tbody>
<tr>
<td>ESR1</td>
<td>35 (50)</td>
<td>17 (18)</td>
</tr>
<tr>
<td>ERBB2</td>
<td>6 (9)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>STK6</td>
<td>34 (49)</td>
<td>30 (39)</td>
</tr>
<tr>
<td>PLAU</td>
<td>10 (14)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>STAT1</td>
<td>4 (6)</td>
<td>6 (8)</td>
</tr>
<tr>
<td>VEGF</td>
<td>7 (10)</td>
<td>4 (5)</td>
</tr>
<tr>
<td>NA</td>
<td>9 (9)</td>
<td>30 (39)</td>
</tr>
</tbody>
</table>

*ESR1* = luminal/basal  
*ERBB2* = Her2-neu  
*STK6* = proliferation/GGI  
*PLAU* = stroma/invasion  
*STAT1* = immune response  
*VEGF* = angiogenesis  
*NA* = undetermined

(Van de Vijver et al. NEJM, 2002)  
(Wang et al. The Lancet, 2005)
SECOND GENERATION GENOMIC SIGNATURES
DO GENOMIC TESTS REPLACE CLASSICAL PROGNOSTIC FACTORS OR ADD TO THEM?

Genomic

Risk factors: Hazard ratio: P-value:
poor-vs-good 3e-05 0.004
grade (2+3 vs 1) 1e-06 0.03
ER-negative 0.05 0.9
node-positive 1e-07 0.2
size >2cm 0.002 0.006
age >50y 0.5 0.5

Clinico-pathological

Untreated

Genomic

Risk factors: Hazard ratio: P-value:
poor-vs-good 2e-09 9e-05
grade (2+3 vs 1) 2e-09 0.1
ER-negative 0.0003 0.01
node-positive 6e-07 1e-06
size >2cm 1e-05 0.003
age >50y 0.3 0.2

Clinico-pathological

Treated

C. Sotiriou et al
PAM 50 ROR (PROSIGNA®)

Parker et al, JCO 2009

- **PAM 50 ROR score** is calculated by using the expression profile of 50 selected genes from 4 intrinsic subtypes, a proliferation score and pathologic tumor size.
- Developed in patients under ET therapy.
- Adds prognostic information, within first 10 yrs of follow up.
PAM 50 ROR (PROSIGNA®) and sensitivity to CT

- Sensitivity of 94% of identifying no responders using the ROR-S model (A)
- Model of ROR-S predictive for pCR (B)

Parker et al, JCO 2009
- 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
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

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.
Subgroups more likely to benefit from long term AI therapy

- Node + (MA17R and DATA)
- Prior chemo (MA17R and DATA)
- Shorter gap without endocrine therapy (MA17R)
- Prior TAM (MA17R and B42)
- Larger tumors (DATA)
- ER AND PR + (DATA)
- Genomic risk (BCI?)

We have to individualize our treatment when it comes to extending AI therapy

JNCI:2013:105:1036-1042
Output: Incremental Cost-Effectiveness Ratio (ICER)

Incremental Cost-Effectiveness Ratio =

Unit cost of treatment – unit cost of comparator

Effect(iveness) of treatment – effect(iveness) of comparator

ICER =

Costs old – Costs new

Effects old – Effects new

Accept technology if ICER < Maximum willingness to pay
<table>
<thead>
<tr>
<th></th>
<th>QALY</th>
<th>costs</th>
<th>iQALY</th>
<th>icosts</th>
<th>ICER</th>
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<tbody>
<tr>
<td><strong>70G</strong></td>
<td>12.44</td>
<td>€28,045</td>
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<tr>
<td><strong>AO</strong></td>
<td>12.20</td>
<td>€26,915</td>
<td>70G</td>
<td>0.24</td>
<td>€1,130</td>
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<td><strong>SG</strong></td>
<td>11.24</td>
<td>€35,475</td>
<td>70G</td>
<td>1.20</td>
<td>-€7,430</td>
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**Results CEA I-retrospective**

**Results CEA II-prospective**

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<thead>
<tr>
<th></th>
<th>QALY</th>
<th>costs</th>
<th>iQALY</th>
<th>icosts</th>
<th>ICER</th>
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<tbody>
<tr>
<td><strong>70G</strong></td>
<td>12.49</td>
<td>€26,786</td>
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</tr>
<tr>
<td><strong>AO</strong></td>
<td>11.88</td>
<td>€29,187</td>
<td>70G</td>
<td>0.62</td>
<td>-€2,401</td>
</tr>
</tbody>
</table>

**Dominant = cheaper and more effective**

*Retèl et al, EJC, 2010*
The test identifies subsets with significantly different * risks of relapse * chances of response

The test is * sensitive * specific * reproducible

BIOMARKER
Ready for use routine use in the clinic?

TECHNICAL VALIDATION

CLINICAL VALIDATION

YES

YES (Different LoE)

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

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

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

HER-2 POSITIVE
CT + anti-HER indispensable

CRUCIAL IMPORTANCE OF HIGH QUALITY PATHOLOGY
HT alone
CT \( \rightarrow \) HT

"No Clear" indication from classical factors; some high & some low risk

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

Breast Cancer

**HER2**

**ER/PGR**

Negative predictive value

**HIGH 95%**

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

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>
</tbody>
</table>
| HER2      | IHC ≥10% cells with complete membrane staining
ISH: number of HER2 gene copies ≥6 or the ratio HER2/chromosome 17 ≥ 2 |
| Ki67      | IHC no final consensus on cut-off around 20% (Ki67 < 10% = low; Ki67 > 30% = high) |

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
Overall survival as a function of response to neoadjuvant PCT

Liedtke C et al, J Clin Oncol, 2008, 26:1275
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
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, FERGI</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>

NGS on BOLERO-2, Phase III study of exemestane + everolimus in HR+ HER2- mBC

PIK3CA Mutations, Pathway Aberration, and Everolimus Efficacy

Hortobagyi G et al., NEJM 2012

Courtesy C. Sotiriou
**PALOMA 1/TRIO 18: Progression-Free Survival (ITT Population): Cohort 1 and Cohort 2**

### Cohort 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Events (%)</th>
<th>Median PFS, months (95% CI)</th>
<th>Hazard Ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAL + LET</td>
<td>15 (44)</td>
<td>26.1 (11.2, NE)</td>
<td>0.299 (0.156, 0.572)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LET</td>
<td>25 (78)</td>
<td>5.7 (2.6, 10.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Cohort 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Events (%)</th>
<th>Median PFS, months (95% CI)</th>
<th>Hazard Ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAL + LET</td>
<td>26 (52)</td>
<td>18.1 (13.1, 27.5)</td>
<td>0.508 (0.303, 0.853)</td>
<td>0.0046</td>
</tr>
<tr>
<td>LET</td>
<td>34 (69)</td>
<td>11.1 (7.1, 16.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cohort 1: ER+/HER2− advanced breast cancer
Cohort 2: ER+/HER2− advanced breast cancer with **CCDN1** amplification and/or loss of p16

**UNSELECTED EVEN BETTER THAN BIOMARKER-DRIVEN!!**
PALOMA 2: Subgroup Analysis of PFS by Biomarker

**Qualitative Analysis**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>n</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>666</td>
<td>0.58 (0.46–0.72)</td>
</tr>
<tr>
<td>ER+</td>
<td>504</td>
<td>0.57 (0.44–0.74)</td>
</tr>
<tr>
<td>ER–</td>
<td>62</td>
<td>0.41 (0.22–0.75)</td>
</tr>
<tr>
<td>Rb+</td>
<td>512</td>
<td>0.53 (0.42–0.68)</td>
</tr>
<tr>
<td>Rb–</td>
<td>51</td>
<td>0.68 (0.31–1.48)</td>
</tr>
<tr>
<td>Cyclin D1+</td>
<td>549</td>
<td>0.56 (0.44–0.71)</td>
</tr>
<tr>
<td>Cyclin D1–</td>
<td>15</td>
<td>1.0 (0.29–3.46)</td>
</tr>
<tr>
<td>p16+</td>
<td>466</td>
<td>0.52 (0.40–0.67)</td>
</tr>
<tr>
<td>p16–</td>
<td>84</td>
<td>0.73 (0.39–1.36)</td>
</tr>
<tr>
<td>Ki-67 ≤20%</td>
<td>318</td>
<td>0.53 (0.38–0.74)</td>
</tr>
<tr>
<td>Ki-67 &gt;20%</td>
<td>235</td>
<td>0.57 (0.41–0.79)</td>
</tr>
</tbody>
</table>

**Quantitative Analysis**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Percentile</th>
<th>n</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>≤25th</td>
<td>142</td>
<td>0.50 (0.32–0.78)</td>
</tr>
<tr>
<td>ER status</td>
<td>&gt;25th to &lt;75th</td>
<td>282</td>
<td>0.53 (0.37–0.74)</td>
</tr>
<tr>
<td></td>
<td>≥75th</td>
<td>142</td>
<td>0.65 (0.41–1.05)</td>
</tr>
<tr>
<td>Rb status</td>
<td>≤25th</td>
<td>154</td>
<td>0.57 (0.36–0.88)</td>
</tr>
<tr>
<td></td>
<td>&gt;25th to &lt;75th</td>
<td>249</td>
<td>0.46 (0.32–0.67)</td>
</tr>
<tr>
<td></td>
<td>≥75th</td>
<td>160</td>
<td>0.63 (0.42–0.95)</td>
</tr>
<tr>
<td>Cyclin D1 status</td>
<td>≤25th</td>
<td>141</td>
<td>0.41 (0.26–0.65)</td>
</tr>
<tr>
<td></td>
<td>&gt;25th to &lt;75th</td>
<td>247</td>
<td>0.69 (0.48–1.00)</td>
</tr>
<tr>
<td></td>
<td>≥75th</td>
<td>176</td>
<td>0.52 (0.34–0.78)</td>
</tr>
<tr>
<td>p16 status</td>
<td>≤25th</td>
<td>140</td>
<td>0.74 (0.46–1.20)</td>
</tr>
<tr>
<td></td>
<td>&gt;25th to &lt;75th</td>
<td>258</td>
<td>0.62 (0.44–0.89)</td>
</tr>
<tr>
<td></td>
<td>≥75th</td>
<td>152</td>
<td>0.33 (0.21–0.52)</td>
</tr>
</tbody>
</table>

HR=hazard ratio; LET=letrozole; PAL=palbociclib; PCB=placebo; PFS=progression-free survival.

R. Finn et al, ESMO 2016
ESR1 mutations seem to be associated with resistance to AIs

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</thead>
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<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

PFS by *ESR1* Mutation Status

**ESR1 positive**

- Palbociclib + Fulvestrant: median PFS 9.4 mo (95% CI: 4.1–11.2)
- Placebo + Fulvestrant: median PFS 4.1 mo (95% CI: 2.8–5.6)

HR 0.524 (95% CI: 0.32–0.87)  
*P* = 0.0052

**ESR1 negative**

- Palbociclib + Fulvestrant: median PFS 9.5 mo (95% CI: 9.2–13.9)
- Placebo + Fulvestrant: median PFS 3.8 mo (95% CI: 3.4–7.4)

HR 0.438 (95% CI: 0.31–0.62)  
*P* < 0.0001

**Interaction P-values**

- *P* = 0.1772
- *P* = 0.4877


N. Turner, ASCO 2016
ESR1 mutations and selection of endocrine therapy

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

Incidence of ESR1-Mutations

- Primary: 1% ESR1-WT, 99% ESR1-Mutant
- Early MBC: 3% ESR1-WT, 92% ESR1-Mutant
- Late MBC: 20% ESR1-WT, 80% ESR1-Mutant

ESR1-WT: No difference between Exemestane and Fulvestrant

- Exemestane: Median PFS, 8.0 months (95% CI, 3.0 to 11.5)
- Fulvestrant-containing regimen: Median PFS, 5.4 months (95% CI, 3.7 to 8.1)

HR, 1.07 (95% CI, 0.68 to 1.67); P = .77

ESR1-Mutant tumours: less sensitive to Exemestane

- Exemestane: Median PFS, 2.6 months (95% CI, 2.4 to 6.2)
- Fulvestrant-containing regimen: Median PFS, 5.7 months (95% CI, 3.0 to 8.5)

HR, 0.52 (95% CI, 0.30 to 0.92); P = .02

ESR1-status makes no difference in response to SERDs


Courtesy Peter Schmid, ESMO 2016, Discussant
In Conclusion:

ER+ $\rightarrow$ Adjuvant ET
HER2+ $\rightarrow$ Adjuvant anti-HER2 therapy

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...