Perfiles de Expresión Génica en Cáncer de Mama. 
Situación actual y vías de desarrollo

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IBIMA
TOPICS

- Introduction: Anatomy versus biology
- Genomic platforms
- What is the value of genomic platforms in breast cancer?
  - Prognostic value
  - Predictive value
  - Real world
- Beyond to avoid adjuvant CHT: biology, biology, biology....
Classical prognosis and predictive factors

- Age
- Grade
- Histological subtypes
- ER/PR and HER2 status
- Vascular invasion
- Tumor margins
Genomic platforms in breast cancer

Endopredict
Mammaprint
Oncotype
Prosigna
# International Options for Genomic Testing

<table>
<thead>
<tr>
<th><strong>Description</strong></th>
<th><strong>oncotype DX</strong></th>
<th><strong>mammoprint</strong></th>
<th><strong>prosigna</strong></th>
<th><strong>Sividon Diagnostics</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis of a 16-gene and 5 controls which generate a risk of recurrence (RS) score</td>
<td>Analysis of a 70-gene expression signature focused on proliferation</td>
<td>Analysis of 50 genes + 8 controls</td>
<td>Analysis of 8 genes + 3 reference</td>
<td></td>
</tr>
<tr>
<td>Analysis includes ER, PR, HER2 status</td>
<td>Suite of tests not included in FDA clearance</td>
<td>PAM50-based algorithm used to calculate Prosigna Score provide risk stratification</td>
<td>Algorithm based calculation of risk of distant recurrence</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Sample Type</strong></th>
<th>Formalin-Fixed, Paraffin-Embedded</th>
<th>Fresh-frozen tissue/FFPE not cleared</th>
<th>Formalin-Fixed, Paraffin-Embedded</th>
<th>Formalin-Fixed, Paraffin-Embedded</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Test Modality</strong></th>
<th>RT-qPCR</th>
<th>Micro-array</th>
<th>nCounter</th>
<th>RT-qPCR</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Population</strong></th>
<th>HR+, Stage 1&amp;2, Node- and Node+ (1-3 Nodes)</th>
<th>US: ER +/-, Stage 1&amp;2, Node- Ex-US: ER +/-, Stage 1&amp;2, Node+ (up to 3 nodes)</th>
<th>HR+, Stage 1&amp;2</th>
<th>HR+, Stage 1&amp;2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excludes HER2</td>
<td>No guidance on HER2 status</td>
<td>Node- and Node+ (1-3 Nodes)</td>
<td>Node- and Node+ (1-3 Nodes)</td>
<td>Node- and Node+ (1-3 Nodes)</td>
</tr>
<tr>
<td>Pre- and Post-menopausal DCIS</td>
<td>Pre- and Post-menopausal</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Report</strong></th>
<th>Recurrence Score</th>
<th>2 risk groups (Low, High)</th>
<th>Intrinsic Subtype</th>
<th>2 risk groups (Low, High)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 risk groups (Low, Intermediate, High)</td>
<td></td>
<td></td>
<td>Prosigna Score</td>
<td></td>
</tr>
<tr>
<td>N0 0 : 3 risk groups (low, Intermediate, high)</td>
<td></td>
<td></td>
<td>N 1-3: 2 risk groups (low, high)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Service Model</strong></th>
<th>Service from single specialized lab</th>
<th>Service from single specialized lab</th>
<th>IVD genomic assay run in high-complexity CLIA lab</th>
<th>IVD genomic assay</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Timeline</strong></th>
<th>10-14 calendar days from sample receipt</th>
<th>10 working days from sample receipt</th>
<th>3 working days from receipt of sample</th>
<th>2 working days from receipt of sample</th>
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</thead>
</table>

<table>
<thead>
<tr>
<th><strong>FDA Clearance</strong></th>
<th>Not FDA cleared or Approved</th>
<th>Mammaprint Yes (Fresh tissue only)</th>
<th>Yes – assay cleared for use with nCounter DX Analysis System</th>
<th>Not FDA cleared or Approved</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Symphony</strong></th>
<th>Mammprint Yes (Fresh tissue only)</th>
<th>Yes – assay cleared for use with nCounter DX Analysis System</th>
<th>Not FDA cleared or Approved</th>
</tr>
</thead>
</table>
**Oncotype DX® 21-Gene Recurrence Score (RS) Assay**

16 Cancer and 5 Reference Genes From 3 Studies

**PROLIFERATION**
- Ki-67
- STK15
- Survivin
- Cyclin B1
- MYBL2

**ESTROGEN**
- ER
- PR
- Bcl2
- SCUBE2

**INVASION**
- Stromelysin 3
- Cathepsin L2

**HER2**
- GRB7
- HER2

**CD68**

**GSTM1**

**BAG1**

**REFERENCE**
- Beta-actin
- GAPDH
- RPLPO
- GUS
- TFRC

RS = + 0.47 x HER2 Group Score
- 0.34 x ER Group Score
+ 1.04 x Proliferation Group Score
+ 0.10 x Invasion Group Score
+ 0.05 x CD68
- 0.08 x GSTM1
- 0.07 x BAG1

<table>
<thead>
<tr>
<th>Category</th>
<th>RS (0 - 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>RS &lt;18</td>
</tr>
<tr>
<td>Int risk</td>
<td>RS 18 - 30</td>
</tr>
<tr>
<td>High risk</td>
<td>RS ≥ 31</td>
</tr>
</tbody>
</table>
Oncotype DX® Clinical Validation: B-14 Results – Distant Recurrence

Distant Recurrence for the three distinct cohorts identified

Proportion without Distant Recurrence

- **RS <18 n = 338**
- **RS 18-30 n = 149**
- **RS ≥31 n = 181**

B-14 Benefit of Tamoxifen
By Recurrence Score Risk Category

Interaction \( P = 0.06 \)

1 The results should not be used to conclude that tamoxifen should not be given to the high-risk group

Trans ATAC: Recurrence Score® Value Is Prognostic in Node-Positive Patients

Node+ (n = 306; both treatment arms)

<table>
<thead>
<tr>
<th>Recurrence Score group</th>
<th>Hazard ratio* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High vs Low</td>
<td>2.7 (1.5-5.1)</td>
</tr>
<tr>
<td>Int vs Low</td>
<td>1.8 (1.0-3.2)</td>
</tr>
</tbody>
</table>

Prospective Validation of a 21-Gene Expression Assay in Breast Cancer


N Engl J Med
November 19, 2015
Kaplan–Meier Estimates in the Analyses of Invasive Disease–free Survival, Freedom from Recurrence of Breast Cancer at a Distant Site, Freedom from Recurrence at Any Site, and Overall Survival.

PlanB: Five-year disease-free survival in per-protocol population (n=2160) (no chemotherapy in pN0-1 RS 0-11)

- 5-Y DFS: 94%
- 5-Y DFS: 94%
- 5-Y DFS: 84%

Recurrent Score Groups:
- 0-11: 94%
- 12-25: 94%
- >25: 84%

p<0.001

WSG GmbH
B-20 Results: Tam vs Tam + Chemo

Low Risk Patients (RS < 18)

- TAM + Chemo: 218, 8 events
- TAM: 135, 4 events

Int Risk Patients (RS 18 - 30)

- TAM + Chemo: 89, 9 events
- TAM: 45, 4 events

High Risk Patients (RS ≥ 31)

- TAM + Chemo: 117, 13 events
- TAM: 47, 18 events

28% absolute benefit from tam + chemo

SWOG 8814: Breast Cancer-Specific Survival of Node-Positive Patients by Treatment and Recurrence Score® Group

- **RS < 18**
  - Stratified log-rank $P = 0.56$ at 10 years
  - 10-yr BCSS
    - T: 92% vs CAF $\rightarrow$ T: 87%
  - No benefit to CAF over time for low Recurrence Score

- **RS 18-30**
  - Stratified log-rank $P = 0.89$ at 10 years
  - 10-yr BCSS
    - T: 70% vs CAF $\rightarrow$ T: 81%
  - Interaction $P = 0.021$

- **RS ≥ 31**
  - Stratified log-rank $P = 0.033$ at 10 years
  - 10-yr BCSS
    - T: 54% vs CAF $\rightarrow$ T: 73%
  - Strong benefit to CAF over time for high Recurrence Score

RS, Recurrence Score result

Five years BCSM by RS (Oncotype)
Tasa de recidiva a distancia a 5 años

Recidivas a distancia por grupo RS: Bajo 20/996, Intermedio 38/812, Alto 28/220
RD 5 años para grupo de riesgo Bajo (RS <18) = 0,8%; 98% tratadas sin QT.
RD 5 años para grupo de riesgo Intermedio (RS 18-30) = 3,2%; 75% tratadas sin QT

MECM, mortalidad específica por cáncer de mama;
Cl, interval de confianza

Stemmer S et al. Presented at SABCS 2015 (Poster P5-08-02)
Recurrence Score en pacientes N1

- RS 0-11: PlanB
- RS 0-17: SWOG 8814, SEER, CLALIT

<table>
<thead>
<tr>
<th># of Positive Lymph Nodes</th>
<th>RS &lt;10 (N=3,519)</th>
<th>RS 10-30 (N=2,300)</th>
<th>RS ≥31 (N=469)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micrometastases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1,544</td>
<td>98.4% (97.4-99.0%)</td>
<td>178</td>
</tr>
<tr>
<td>2</td>
<td>458</td>
<td>97.1% (91.3-99.0%)</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>139</td>
<td>96.1% (87.0-96.2%)</td>
<td>29</td>
</tr>
<tr>
<td>4+</td>
<td>129</td>
<td>92.9% (73.5-92.2%)</td>
<td>39</td>
</tr>
<tr>
<td>5</td>
<td>72</td>
<td>89.8% (69.9-91.9%)</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>87.6% (65.2-95.7%)</td>
<td>21</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>83.0% (69.5-91.9%)</td>
<td>17</td>
</tr>
<tr>
<td>8+</td>
<td>11</td>
<td>73.5% (53.7-92.8%)</td>
<td>12</td>
</tr>
</tbody>
</table>

- 5-year BCSS outcomes for those with RS <18 ranged from 98.0% (95% CI, 97.4-99.0) in those with micrometastases to 92.8% (95% CI, 73.5-98.2) among those with 4+ positive lymph nodes (Table 2).

Roberts MC, Breast Ca Res Treat, 2017

Petkov et al. SABCS 2016
Schema: TAILORx

Node-Neg, ER-Pos Breast Cancer

Register Specimen banking

Oncotype DX® Assay

RS 11-25
Randomize Hormone Rx vs Chemotherapy + Hormone Rx

RS ≤10 Hormone Therapy Registry

RS >25 Chemotherapy + Hormone Rx

Primary study group
Rxspander: Schema and Patient Flow

Node-positive (1-3 nodes) HR-positive and HER2-negative breast cancer

(N= 8,800)
Patients consent to study-sponsored RS testing, discussion of potential trials, tumor tissue submission and linkage to cancer registry data

STEP 1 REGISTRATION
Tumor tissue submission for RS

(RECURRENCE SCORE

RS > 25
(N= 3,800)
Discuss alternative trials for high risk patients

RS < 25
(N= 5,600)
Physician and patients discuss randomization knowing the RS

Accept

RS < 25
(N= 5,600)
Physician and patients discuss randomization knowing the RS

Accept

Refuse

RS > 25
(N= 3,800)
Discuss alternative trials for high risk patients

RS < 25
(N= 5,600)
Physician and patients discuss randomization knowing the RS

Accept

Refuse

N= 1,600
Record chosen therapy and followed for vital status through cancer registry

STEP 2 REGISTRATION
RANDOMIZATION

N= 4,000
Randomization stratified by
1. RS 0-13 vs. 14-25
2. Menopausal status
3. Axillary node dissection vs. Sentinel node biopsy

N= 2,000
Chemotherapy; appropriate endocrine therapy

N= 2,000
No Chemotherapy; appropriate endocrine therapy

(N= 600)
RS already Available
Physician and patients discuss randomization knowing the RS

N= 8,800
Patients consent to study-sponsored RS testing, discussion of potential trials, tumor tissue submission and linkage to cancer registry data

(N= 3,800)
Discuss alternative trials for high risk patients

N= 5,600
Physician and patients discuss randomization knowing the RS

Accept

Refuse

N= 1,600
Record chosen therapy and followed for vital status through cancer registry
A signature consisting of 70 significant prognosis genes was identified

Supervised classification prognosis
Leave-one-out cross-validation

Threshold set with 10% false negatives
91% sensitivity, 73% specificity

Development of 70 gene expression profile

Tumor samples of known clinical outcome

Unbiased full genome gene expression analysis

Prognosis reporter genes

Distant metastases group

No distant metastases group

70 prognosis genes

~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
MammaPrint: Improved Clinical Management Profiling vs St Gallen selection

MammaPrint: 40% in good profile 60% in poor profile

St Gallen: 15% in low risk 85% in high risk

MammaPrint improved prediction and more accurate

Chi² = 24.96, P = 5.86e-007

Chi² = 3.77, P = 0.0521

MammaPrint prognosis in postmenopausal patients

KM survival curve: Breast cancer > 55 year old

Overall survival HR 2.3, 0.95 CI [1.3-4.1], p=0.0049

Good prognosis profile

Poor prognosis profile

150 patients
MammaPrint performance in patients with 1-3 positive node(s)

N=241 breast cancer patients with 1-3 positive lymph node(s)

Milan & NKI

Multivariate HR 6.59 (95% CI 1.71 to 25.45; p = 0.006)
Diseño del estudio MINDACT

Diagnosis of breast cancer
Screening informed consent

Surgery

Local pathology
(T1-3, 0 to 3 positive nodes, ER status, HER2 status)

Agendia
(frozen tumor sample shipment, RNA extraction, microarray analysis)

Enrollment

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

No Chemotherapy

R-T

Chemotherapy

c-High/g-High
### Características de los pacientes por grupos

<table>
<thead>
<tr>
<th></th>
<th>LOW RISK (no chemo)</th>
<th>OVERTREATMENT GROUP</th>
<th>UNDERTREATMENT GROUP</th>
<th>HIGH RISK (chemo)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (median)</strong></td>
<td>55</td>
<td>55</td>
<td>55</td>
<td>53</td>
</tr>
<tr>
<td><strong>ER+</strong></td>
<td>99.9%</td>
<td>97.4%</td>
<td>90.4%</td>
<td>60.2%</td>
</tr>
<tr>
<td><strong>IHC Subtype</strong></td>
<td>96% Luminal • HER2+ : 4%</td>
<td>91% Luminal • HER2+ : 8% • Triple Neg : 1%</td>
<td>79% Luminal • HER2+ : 12% • Triple Neg : 9%</td>
<td>50% Luminal • HER2+ : 19% • Triple Neg : 31%</td>
</tr>
<tr>
<td><strong>Grade</strong></td>
<td>Grade 1 or 2 : 98%</td>
<td>Grade 2 or 3 : 94%</td>
<td>Grade 1 or 2 : 85%</td>
<td>Grade 3 : 76%</td>
</tr>
<tr>
<td><strong>Node Positive</strong></td>
<td>6%</td>
<td>48%</td>
<td>2%</td>
<td>26%</td>
</tr>
<tr>
<td><strong>Tumor Size &gt;2cm</strong></td>
<td>4%</td>
<td>58%</td>
<td>2%</td>
<td>48%</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>Assigned: No Chemotherapy Compliance=99% • Endocrine Therapy=79%</td>
<td>Randomized: No Chemotherapy Compliance=89%</td>
<td>Randomized: No Chemotherapy Compliance=86% • Endocrine Therapy=94% Trastuzumab=5%</td>
<td>Assigned: Chemotherapy Compliance=96% Endocrine Therapy=59% Trastuzumab=15%</td>
</tr>
</tbody>
</table>
Grupos concordantes: DMFS a 5 años

Distant Metastasis Free Survival

% at 5 year

\[ \begin{align*}
\text{cL/gL} & \quad 97.6 \ (96.9, \ 98.1) \\
\text{cH/gH} & \quad 90.6 \ (89.0, \ 92.0)
\end{align*} \]

Number of patients at risk:

\[ \begin{array}{cccc}
\text{O} & \text{N} & \text{Corrected risk} \\
77/2744 & 2627/2330 & 735/33 & 11/1806
\end{array} \]
Objetivo primario: DMFS a 5 años cH/gL sin QT

Ho: DMFS cHgL sin Quimioterapia = 92%

DMFS cHgL observada sin QT = 94,7%
95% IC (92,5% – 96,2%)...........excluye 92%
Se rechaza Ho

cHgL PUEDEN EVITAR LA QT
SIN AFECTAR LA DMFS
The EP and EPclin Signatures


- EP was determined using qRT-PCR and formalin-fixed, paraffin-embedded tissue samples.

- EP signature was trained on samples from tamoxifen (TAM)-treated women (n = 964).

- EP signature was validated using samples from patients on the Phase III ABCSG-6 (n = 378) and 8 (n = 1,324) trials.

- EPclin is a predefined score incorporating EP, tumor size and nodal status.

<table>
<thead>
<tr>
<th>Reference genes</th>
<th>Member 1</th>
<th>Member 2</th>
<th>Member 3</th>
<th>Member 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BIRC5</td>
<td>UBE2C</td>
<td>AZGP1</td>
<td>MGP</td>
</tr>
<tr>
<td></td>
<td>RBBP8</td>
<td>IL6ST</td>
<td>DHCR7</td>
<td>STC2</td>
</tr>
</tbody>
</table>

Distant Metastasis-Free Survival (DMFS)

- EP low-risk group (49% of pts) had a significantly improved clinical outcome before and after 5 y of follow-up:
  - 96.3% were distant metastasis free between 5 and 10 y

With permission from Dubsky P et al. Proc SABCS 2012;Abstract S4-3.
EP Score versus EPclin Score

EP low risk (49% of pts) >5 y

Absence of distant recurrence

$P$ (log rank) = 0.002
Hazard ratio: 2.91 (1.98–4.27)

96.3% pts were distant metastasis free at 5-10 y

EPclin low risk (64% of pts) >5 y

Absence of distant recurrence

$P$ (log rank) < 0.001
Hazard ratio: 5.11 (3.48–7.51)

>98% pts were distant metastasis free at >5 y

With permission from Dubsky P et al. Proc SABCS 2012;Abstract S4-3.
# PAM50 Defined Intrinsic Subtype: Universal Language of Breast Cancer

## Independently Validated

PAM50-based intrinsic subtyping has been independently validated by TCGA and published in *Nature* in September 2012.

## Endorsed in 2013 St. Gallen Guidelines

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Therapy Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>Endocrine therapy alone</td>
</tr>
<tr>
<td>Luminal B</td>
<td>If HER2-, endocrine +/- cytotoxic therapy&lt;br&gt;If HER2+, cytotoxics + anti-HER2 + endocrine&lt;br&gt;Could include anthracyclines and taxanes</td>
</tr>
<tr>
<td>HER2 enriched</td>
<td>Cytotoxics + anti-HER2&lt;br&gt;Could include anthracyclines and taxanes</td>
</tr>
<tr>
<td>Basal-like</td>
<td>Cytotoxic therapy alone, potentially including anthracyclines, taxanes, and alkylating agent&lt;br&gt;Do not routinely use cisplatin or carboplatin</td>
</tr>
</tbody>
</table>

## References

Prosigna Results are Based on Clinical and Genomic Information

- Gene expression data are weighted with clinical variables to determine an integer score from 0 through 100 (Prosigna Score) indicative of the probability of distant recurrence.

- Prosigna/ROR Score is based on the similarity of the gene expression profile to PAM50 genomic profiles and:
  - proliferation score
  - gross tumor size

PAM50 determines genomic profile

Clinical Features

Nodal Status =
- Node 0
- Node 1-3

Gross Tumor Size =
- <2cm
- ≥2cm

Proprietary Algorithm

\[
\text{Prosigna Score} = aR_{\text{LumA}} + bR_{\text{LumB}} + cR_{\text{Her2}} + dR_{\text{Basal}} + eP + fT
\]

Pearson’s correlation to centroids

- Proliferation Score
- Gross Tumor Size
Prosigna Clinically Validated in Two Studies

**TransATAC Study**
- N = 1,017 patients
- Compared Identical RNA
- *Published in Journal of Clinical Oncology, July 2013*

**ABCSG8 Study**
- N = 1,620 patients
- Presented at San Antonio Breast Cancer Symposium in December 2012
- *Published in Annals of Oncology Dec. 2013*

Prosigna’s clinical performance has been validated by two prospectively defined analysis, registration-quality studies with ≥10-yr median follow-up in >2400 postmenopausal women with HR+ early stage breast cancer treated with endocrine therapy alone.

- **Primary Objective**: Validate published observations that Prosigna/ROR Score provides additional prognostic information over and above standard clinical variables for DRFS at 10 yrs
  - **Primary Analysis**: All patients
  - **Secondary Analysis**: Node-negative, node-positive, and HER2-negative patients
- **Secondary Objective**: Validate observations that Luminal A and Luminal B patients have statistically significantly different DRFS at 10 yrs

Findings From the Combined Analysis of >2400 Patient Samples

**Prosigna Low Risk patients remain Low Risk over 10yrs**

**DRFS by Risk-Group for Node-negative Patients**

- Low-risk
- Intermediate-risk
- High-risk

<table>
<thead>
<tr>
<th>Follow-Up Time (yrs)</th>
<th>Percent Without Distance Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>6</td>
<td>0.7</td>
</tr>
<tr>
<td>8</td>
<td>0.6</td>
</tr>
</tbody>
</table>

**Follow-Up Time (yrs)**

- 0
- 2
- 4
- 6
- 8
- 10

**Percent Without Distance Recurrence**

- 1.0
- 0.9
- 0.8
- 0.7
- 0.6

**Risk Group**

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>N (%)</th>
<th>Events Through 10 Yrs</th>
<th>% Without Recurrence at 10 yrs [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>875 (49%)</td>
<td>31</td>
<td>96.2% [94.7% - 97.3%]</td>
</tr>
<tr>
<td>Intermediate</td>
<td>551 (31%)</td>
<td>53</td>
<td>89.2% [86.1% - 91.7%]</td>
</tr>
<tr>
<td>High</td>
<td>360 (20%)</td>
<td>73</td>
<td>77.7% [72.8% - 81.95%]</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1,786 (100%)</strong></td>
<td><strong>157</strong></td>
<td></td>
</tr>
</tbody>
</table>

**DRFS by Risk-Group for Node-positive Patients (1-3 positive nodes)**

- Low-risk
- Intermediate-risk
- High-risk

<table>
<thead>
<tr>
<th>Follow-Up Time (yrs)</th>
<th>Percent Without Distance Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>6</td>
<td>0.7</td>
</tr>
<tr>
<td>8</td>
<td>0.6</td>
</tr>
</tbody>
</table>

**Follow-Up Time (yrs)**

- 0
- 2
- 4
- 6
- 8
- 10

**Percent Without Distance Recurrence**

- 1.0
- 0.9
- 0.8
- 0.7
- 0.6

**Risk Group**

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>N (%)</th>
<th>Events Through 10 yrs</th>
<th>% Without Recurrence at 10 yrs [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>24 (4%)</td>
<td>2</td>
<td>91.7% [70.6% - 97.8%]</td>
</tr>
<tr>
<td>Intermediate</td>
<td>211 (36%)</td>
<td>18</td>
<td>90.4% [85.2% - 93.9%]</td>
</tr>
<tr>
<td>High</td>
<td>355 (60%)</td>
<td>87</td>
<td>71.8% [66.3% - 76.6%]</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>590 (100%)</strong></td>
<td><strong>107</strong></td>
<td></td>
</tr>
</tbody>
</table>

Knowledge of Luminal Status May Improve Disease Management

**DRFS by Risk-Group for Node-Negative Patients**

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>N (%)</th>
<th>Events Through 10 yrs</th>
<th>% Without Recurrence at 10 yrs [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>1254</td>
<td>62</td>
<td>94.6% [93.1% – 95.8%]</td>
</tr>
<tr>
<td>Luminal B</td>
<td>460</td>
<td>75</td>
<td>81.9% [77.7% – 85.3%]</td>
</tr>
<tr>
<td>Total</td>
<td>1,714</td>
<td>137</td>
<td></td>
</tr>
</tbody>
</table>

**DRFS by Risk-Group for Node-Positive Patients**

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>N (%)</th>
<th>Events Through 10 yrs</th>
<th>% Without Recurrence at 10 yrs [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>375</td>
<td>41</td>
<td>87.6% [83.5% – 90.8%]</td>
</tr>
<tr>
<td>Luminal B</td>
<td>186</td>
<td>52</td>
<td>68.3% [60.4% – 75.0%]</td>
</tr>
<tr>
<td>Total</td>
<td>561</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>

### Luminal A vs Luminal B

<table>
<thead>
<tr>
<th></th>
<th>HR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A (N=1530 (71.6%))</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Luminal B (N=542 (25.4%))</td>
<td>2.89 (2.07- 4.02)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Sestak et al. SABCS 2013 and JCO 2014
Response rates according to intrinsic subtype classification

Fraction Responding to NAC (pCR, M&P Score =5)

Intrinsic Subtype

BASAL LIKE | HER2 ENRICHED | LUMINAL A | LUMINAL B

Chica Parrado R. et al. SABCS 2015
Chemotherapy (CT) and hormonotherapy (HT) as neoadjuvant treatment in luminal breast cancer patients: results from the GEICAM/2006-03, a multicenter, randomized, phase-II study

E. Alba¹, L. Calvo², J. Albanelli³, J. R. De la Haba⁴, A. Arcusa Lanza⁵, J. I. Chacon⁶, P. Sanchez-Rovira⁷, A. Plazaola⁸, J. A. Lopez Garcia-Asenjo⁹, B. Bermejo¹⁰, E. Carrasco¹¹, & A. Lluch¹⁰ on behalf of GEICAM

N=95 patients ER+/PR+/HER2- and KRT8/18+

The clinical response rate was.

66% (95% CI: 52.5–79.5%) with CT

48% (95% CI: 33.8–62%) with HT

Exploratory P-value = 0.075.
Association between chemo/endocrine-sensitivity, ROR (prognosis) and PAM50 subtypes (biology)

N=7,100 primary tumors

Pearson Correlation Coeff. CESP vs. ROR = -0.70
**UK OPTIMA-Preliminary Trial: Concordance between Assays**

**Concordance by Risk**

<table>
<thead>
<tr>
<th>Kappa statistic (95% CI)</th>
<th>MammaPrint (Low)</th>
<th>ROR_PT (Low/int)</th>
<th>IHC4 (Low/int)</th>
<th>IHC4-AQUA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrence Score® result ≤25 (OPTIMA low-risk)</td>
<td>0.40 (0.30-0.49)</td>
<td>0.44 (0.33-0.54)</td>
<td>0.53 (0.41-0.65)</td>
<td>0.40 (0.30-0.51)</td>
</tr>
<tr>
<td>MammaPrint</td>
<td>-</td>
<td>0.53 (0.43-0.63)</td>
<td>0.33 (0.21-0.44)</td>
<td>0.42 (0.30-0.53)</td>
</tr>
<tr>
<td>ROR_PT (Low/int)</td>
<td>-</td>
<td>-</td>
<td>0.39 (0.27-0.50)</td>
<td>0.43 (0.31-0.54)</td>
</tr>
<tr>
<td>IHC4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.60 (0.50-0.70)</td>
</tr>
</tbody>
</table>

- Only moderate agreement existed between tests (kappa values for risk assignment range 0.33–0.60). Risk assignment by all 5 tests agreed for only 119 (39%) tumours with 31% agreement for 4/5 tests and subtype assignment by all 3 tests for 179 (59%) tumours.

**Cada plataforma puede clasificar de forma diferente a una misma paciente**

JMS Bartlett et al. Presented at SABCS 2014 (Poster P4-11-07)
RC Stein et al. Presented at ECC 2015 (Abstract 1809)
Biological Processes Associated with Breast Cancer Clinical Outcome Depend on the Molecular Subtypes

Christine Desmedt,1 Benjamin Haibe-Kains,1,2 Pratyaksha Wirapati,3,4 Marc Buyse,5 Denis Larsimont,1 Gianluca Bontempi,2 Mauro Delorenzi,3,4 Martine Piccart,1 and Christos Sotiriou1

Most published signatures are not significant better predictors than random signatures of identical size.
Meta-PCNA adjustment decreases the prognostic abilities of published signatures

Intrinsic subtype distribution within clinically HER2- and HER2+ disease

HER2-positivity enriches 6.62-fold for the HER2E subtype and diminishes 3.65-fold and 1.66-fold for the Luminal A and Basal-like subtypes, respectively.

The proportion of Luminal B tumors based on cHER2 status was not significantly different.

Prat et al. JNCI 2014
The HER2-enriched subtype is associated with higher responses and improved survival outcomes in HER2+ breast cancer in the NOAH study.

Chemotherapy: 
AT x 3 $\rightarrow$ T x 4 $\rightarrow$ CMF x 3

156 (46.7%) pre-treatment samples were PAM50 profiled.

### RESPONSE

<table>
<thead>
<tr>
<th>Subtype</th>
<th>pCR</th>
<th>Odds Ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2+/HER2-E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No-trastuzumab</td>
<td>27.6%</td>
<td>5.1</td>
<td>0.009</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>52.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2+/nonHER2-E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No-trastuzumab</td>
<td>18.2%</td>
<td>2.1</td>
<td>0.352</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>34.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2+/HR-/HER2-E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No-trastuzumab</td>
<td>25.0%</td>
<td>8.7</td>
<td>0.002</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>63.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2+/HR-/nonHER2-E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No-trastuzumab</td>
<td>11.1%</td>
<td>2.4</td>
<td>0.582</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>31.3%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: interaction test between HER2E subtype and treatment was not statistically significant.

Treatment implications of molecular heterogeneity of human epidermal growth factor receptor 2 (HER2) – positive breast cancer

Carey LA et al. JCO 2016
pCR rates according to PAM50 intrinsic subtypes (normal-like excluded)

<table>
<thead>
<tr>
<th>PAM50 intrinsic subtype</th>
<th>N tot</th>
<th>pCR; n (%)</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Her2-enriched</td>
<td>22</td>
<td>11 (50)</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>Basal-like</td>
<td>12</td>
<td>3 (25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal B</td>
<td>14</td>
<td>3 (21.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal A</td>
<td>21</td>
<td>2 (9.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>19 (27.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$N$, $n$, number; tot, total; pCR, pathological complete response; $P$, $P$-value.
Response rates according to Prosigna subtype classification

Intrinsic Subtype within HER2+ (IHC)

- HER2 ENRICHED
- LUMINAL A
- LUMINAL B

Fraction Responding to NAC (pCR, M&P Score =5)

Responders
Non-Responders

Santonja A. et al. SABCS 2015
Molecular Characterization of Basal-Like and Non-Basil-Like Triple-Negative Breast Cancer


Translational Genomics Unit, Breast Cancer Unit, and Medical Oncology Department, Vall d’Hebron Institute of Oncology, Barcelona, Spain; Lineberger Comprehensive Cancer Center, Department of Genetics, and Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, North Carolina, USA

<table>
<thead>
<tr>
<th>Molecular Type</th>
<th>Basal-like</th>
<th>HER2-enriched</th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>Normal-like</th>
<th>Claudin-low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal-like</td>
<td>23.3%</td>
<td>4.4%</td>
<td>2.9%</td>
<td>7.8%</td>
<td>7.0%</td>
<td>2.2%</td>
</tr>
<tr>
<td>HER2-enriched</td>
<td>78.6%</td>
<td>4.4%</td>
<td>2.9%</td>
<td>7.8%</td>
<td>7.0%</td>
<td>2.2%</td>
</tr>
</tbody>
</table>
Triple-Negative vs. Basal-Like: Definitions

- **Triple negative but not basal**
  - Definition by IHC
  - Includes other histologies (medullar, adenoid cystic)
  - 10-30% can also include “claudin-low,” a subtype notable for high expression of stem cell markers
  - 85% of TNBC do not have BRCA or other deleterious mutations

- **Basal but not triple negative**
  - Definition by gene expression
  - Includes most BRCA1 mutated tumors
  - 15-40% are ER+, PR+ or HER2+

**Triple Negative**

- ~15% of all breast carcinomas
- Poorly differentiated
- Express CK 5/6, 17, EGFR (+)

**Basal**

- ~75% of TNBC have Basal gene expression

**BRCA 1-2**

- BRCA1-2 mutated tumors
- ~5% of Breast Cancer
- 60-80% BRCA-1 carriers are basal-like

Lakhani Sr J Clin Oncol 2002
Atchley DP J Clin Oncol 2008
Couch F J Clin Oncol 2014
Objective response – Basal-like (Prosigna PAM50)

---

Graph showing the percentage of patients with an objective response (OR) at cycle 3 or 6, with 95% CI, for Basal-like and Non Basal-like patients, treated with Carboplatin and Docetaxel.
In Lehmann et al., 7 “subtypes” were identified by analyzing global gene expression analyses in TNBC-only.

**Basal 1**: cell cycle
**Basal 2**: cell cycle + growth factor
**Immunomodulatory**: immune
**Mesenchymal**
**Mesenchymal Stem Like**
**Luminal Androgen Receptor**
**Unclassifiable**
Comparisons to previous subtypes: PAM50 intrinsic subtypes and TNBC types by four (4) clusters
Resultados

RCp población estudio vs RCp Subtipos de Lehmann

Consiguen RCp el 30% de las pacientes con CMTN

Pacientes BL1 consiguen un 50% de RCp mientras que las pacientes LAR consiguen un 11% (p>0,05)
LAR subtype and pCR
CONCLUSIONES

- El cáncer de mama es una enfermedad heterogénea más allá de los factores anatómicos clásicos y de la clasificación IHC.
- Existen cuatro plataformas actualmente comercializadas para su uso en cáncer de mama.
- Todas las plataformas definen poblaciones con pronósticos diferentes.
- Todas las plataformas disponibles, a pesar de estar construidas a partir de sets de genes diferentes, capturan el mismo fenómeno biológico: proliferación celular.
- El valor predictivo con respecto a la hormonosensibilidad y quimiosensibilidad de las diferentes plataformas se deriva del análisis de estudios retrospectivos.
- PAM50 es la única plataforma que aporta información biológica.
- PAM50 define a una población HER2E en la que probablemente el tratamiento antiHER2 es más eficaz (no útil clínicamente todavía).
- PAM50 define el subtipo no basal-like dentro del TN en el que las sales de platino no son eficaces.
Single Gene Expression association with “Chemo vs. Endocrine Sensitivity”

• Genes associated with chemotherapy response are basically proliferation-related genes (i.e. TOP2A, EXO1, CKS2, CEP55)

• Genes associated with endocrine therapy response are luminal-related genes (i.e. AR, FOXA1, CA12) and mesenchymal (i.e. ZEB1, AXL, VEGFR, FAP, TGFBR2). High expression of FGFR1 is also associated with endocrine therapy response.
Logistic Regression: Her2E subtype correlation and probability of response to NAC + HER2 targeted therapy

Odds ratio [Unit increase of 1 in Her2E correlation] = 88.2, P-value = 0.004
TNBC: PAM50 (Nanostring nCounter) y Array HTA2.0 (Affymetrix)
Predicting Response and Survival in Chemotherapy-Treated Triple-Negative Breast Cancer

Interaction tests: Luminal A $p=0.023$; Proliferation Score $p=0.005$

*Each variable is adjusted for Age, Grade, T.size, Nodal status

Prat, Lluch, Albanell et al. BJC 2014
# Characteristics of genomic platforms commercially available

<table>
<thead>
<tr>
<th>Platform</th>
<th>What does measure?</th>
<th>Endorsement or recommendation</th>
<th>HT predictive value (RCT)</th>
<th>CHT predictive value (RCT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endopredict</td>
<td>Prognosis (metástasis in ER+/HER2-PN₀/N⁺)</td>
<td>CE mark</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Mammaprint</td>
<td>Prognosis (metástasis 5 y. in pN₀)</td>
<td>FDA</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Oncotype</td>
<td>Prognosis (recurrence in ER+, pN₀ treated with TMX)</td>
<td>NCCN, ASCO, St. Gallen</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Prosigna</td>
<td>Prognosis BIOLOGY</td>
<td>CE mark</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
Probability of response to NAC

**Luminal A Subtype Correlation**

**Luminal B Subtype Correlation**

**HER2 Enriched Subtype Correlation**

**BasalLike Subtype Correlation**
Differentiated: Fewer Intermediate Risk Node-Negative Patients

TransATAC Study Comparing Prosigna and Oncotype DX

Patient Distribution & Kaplan-Meier curves by risk groups

Node-negative patients only. Clinical-pathological variables excluded. N = 739

1 Risk groups prospectively defined based on predicted probability of 10 year distant recurrence (Low <10%, Intermediate 10-20%, High >20%). Clinical and pathological variables excluded from analysis.

Testing the predictive ability of CESP in 675 patients with HR+ disease

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MDACC-based</th>
<th>Málaga-based</th>
<th>Marsden-based</th>
<th>Edinburgh-based</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Num. HR+/HER2-negative</td>
<td>272</td>
<td>-</td>
<td>180</td>
<td>-</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>50.1</td>
<td>-</td>
<td>50.0</td>
<td>-</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-menopausal</td>
<td>NA</td>
<td>-</td>
<td>108</td>
<td>60%</td>
</tr>
<tr>
<td>Post-menopausal</td>
<td>NA</td>
<td>-</td>
<td>72</td>
<td>40%</td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0-T1</td>
<td>19</td>
<td>7%</td>
<td>18</td>
<td>10%</td>
</tr>
<tr>
<td>T2</td>
<td>142</td>
<td>52%</td>
<td>115</td>
<td>67%</td>
</tr>
<tr>
<td>T3-T4</td>
<td>111</td>
<td>41%</td>
<td>39</td>
<td>23%</td>
</tr>
<tr>
<td>Tumor size (mean)</td>
<td>NA</td>
<td>-</td>
<td>3.8</td>
<td>4.2</td>
</tr>
<tr>
<td>Node</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>96</td>
<td>35%</td>
<td>67</td>
<td>37%</td>
</tr>
<tr>
<td>N1</td>
<td>133</td>
<td>49%</td>
<td>61</td>
<td>34%</td>
</tr>
<tr>
<td>N2-N3</td>
<td>43</td>
<td>16%</td>
<td>52</td>
<td>29%</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>28</td>
<td>12%</td>
<td>27</td>
<td>19%</td>
</tr>
<tr>
<td>G2</td>
<td>136</td>
<td>60%</td>
<td>96</td>
<td>68%</td>
</tr>
<tr>
<td>G3</td>
<td>91</td>
<td>40%</td>
<td>46</td>
<td>32%</td>
</tr>
</tbody>
</table>
Association between chemo-sensitivity, endocrine-sensitivity, ROR and PAM50 subtypes

N=1,734 primary tumors from METABRIC (Nature 2012). All Subtypes Represented.

<table>
<thead>
<tr>
<th>PAM50 ROR GROUPS</th>
<th>PAM50 TREATMENT SENSITIVITY GROUPS</th>
<th>PAM50 Subtype Call</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ENDOCRINE</td>
<td>CHEMO</td>
<td>LumA</td>
</tr>
<tr>
<td>LOW ROR</td>
<td>HIGH</td>
<td>LOW</td>
<td>188</td>
</tr>
<tr>
<td></td>
<td>INTERM</td>
<td>INTERM</td>
<td>11</td>
</tr>
<tr>
<td>MED ROR</td>
<td>HIGH</td>
<td>LOW</td>
<td>179</td>
</tr>
<tr>
<td></td>
<td>INTERM</td>
<td>INTERM</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>LOW</td>
<td>HIGH</td>
<td>12</td>
</tr>
<tr>
<td>HIGH ROR</td>
<td>INTERM</td>
<td>INTERM</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>LOW</td>
<td>HIGH</td>
<td>-</td>
</tr>
</tbody>
</table>