PROGNOSTIC AND PREDICTIVE MARKERS FOR BREAST CANCER MANAGEMENT

ESMO Preceptorship Programme
Breast Cancer
Multidisciplinary management, standards of care, therapeutic targets and future perspectives
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Prof. Fernando Schmitt
Director of Department of Pathology and Medicine
Laboratoire National de Santé, Luxembourg
General-Secretary of the International Academy of Cytology
Role of the Pathologist in Breast Cancer

Aim 1: **Diagnosis**
- Histological type
- Molecular type

Aim 2: **Prognosis**
- Histological type
- Histological grading (plus size and LN status)
- Molecular type

Aim 3: **Prediction**

Predictive factor is defined as a specific patient or tumor characteristic that correlate with response or lack of response to a specific treatment.
OPTIMAL BREAST CANCER PATHOLOGY EVALUATION

- Tumor type
- Tumor size
- Tumor grade
- Lymph node status
- Operative margins
- Peritumoral vascular invasion
- Multifocality/ multicentricity
- Staging pTpNpM R
- Hormone receptor status
- HER2 status
- Ki67 labeling index
- Gene expression profiling and mutational analyzes
- Bio-banking
Nottingham Prognostic Index in Triple-Negative Breast Cancer: a reliable prognostic tool?

<table>
<thead>
<tr>
<th>Tumour Size</th>
<th>TNBC</th>
<th>Non-TNBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>8.2%</td>
<td>27.6%</td>
</tr>
<tr>
<td>T2</td>
<td>65.6%</td>
<td>58.9</td>
</tr>
<tr>
<td>T3</td>
<td>26.2%</td>
<td>13.5%</td>
</tr>
</tbody>
</table>

P < 0.001

Table 3: Association of tumour size and lymph node status in triple-negative and non-triple-negative breast cancer

<table>
<thead>
<tr>
<th>Tumour Size</th>
<th>LNS</th>
<th>Non-Triple Negative Breast Cancer</th>
<th>Triple Negative Breast Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>1 &lt; LNS &lt; 3</td>
<td>LNS &gt; 3</td>
</tr>
<tr>
<td>Tumour Size &lt; 2 cm</td>
<td>66.7%</td>
<td>22.7%</td>
<td>10.7%</td>
</tr>
<tr>
<td>Tumour Size 2-5 cm</td>
<td>39.4%</td>
<td>26.1%</td>
<td>34.5%</td>
</tr>
<tr>
<td>Tumour Size &gt; 5 cm</td>
<td>19.4%</td>
<td>33.3%</td>
<td>47.2%</td>
</tr>
</tbody>
</table>

Statistics:
- (N = 276) P-value* < 0.0001
- (N = 145) P-value* < 0.001
Breast cancer classification and prognosis
Reappraisal of immunohistochemical profiling of special histological types of breast carcinomas: a study of 121 cases of eight different subtypes

César Augusto Alvarenga,¹,² Paula Itagyba Paravidino,¹,² Marcelo Alvarenga,² Madalena Gomes,¹ Rozany Dufloth,³ Luiz Carlos Zeferino,⁴ José Vassallo,⁵,⁶ Fernando C Schmitt¹,⁷

Table 3  Immunohistochemical surrogate profile for the molecular classification of special types of breast carcinoma

<table>
<thead>
<tr>
<th>Molecular classification</th>
<th>Tubular n (%)</th>
<th>Mucinous n (%)</th>
<th>Papillary n (%)</th>
<th>Micropapillary n (%)</th>
<th>Medullary n (%)</th>
<th>Metaplastic n (%)</th>
<th>Apocrine n (%)</th>
<th>Mixed n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>14 (87.5)</td>
<td>13 (46.1)</td>
<td>6 (66.7)</td>
<td>3 (37.5)</td>
<td>-</td>
<td>-</td>
<td>2 (8.3)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>2 (12.5)</td>
<td>14 (51.9)</td>
<td>3 (33.3)</td>
<td>4 (50)</td>
<td>3 (14.3)</td>
<td>1 (10)</td>
<td>8 (33.3)</td>
<td>4 (66.6)</td>
</tr>
<tr>
<td>HER2-overexpression</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (12.5)</td>
<td>-</td>
<td>-</td>
<td>7 (29.2)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Basal-like</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>18 (85.7)</td>
<td>9 (90)</td>
<td>7 (29.2)</td>
<td>-</td>
</tr>
</tbody>
</table>

Breast cancer classification and prognosis
Breast cancer prognostic classification in the molecular era: the role of histological grade

Emad A Rakha¹, Jorge S Reis-Filho², Frederick Baehner³, David J Dabbs⁴, Thomas Decker⁵, Vincenzo Eusebi⁶, Stephen B Fox⁷, Shu Ichihara⁸, Jocelyne Jacquemier⁹, Sunil R Lakhani¹⁰, José Palacios¹¹, Stuart J Schnitt¹², Fernando C Schmitt¹³, Puay-Hoon Tan¹⁴, Gary M Tse¹⁵, Sunil Badve¹⁶ and Ian O Ellis*¹¹

- Tubule formation
- Nuclei pleomorphism
- Mitotic index
**Nottingham Prognostic Index in Triple-Negative Breast Cancer: a reliable prognostic tool?**

<table>
<thead>
<tr>
<th>Molecular Subtype</th>
<th>Tumour Size Mean ± St. Error</th>
<th>Tumour Grade</th>
<th>Lymph Node Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal</td>
<td>2.92 cm ± 0.105 (N = 302)</td>
<td>24.1%</td>
<td>26.7%</td>
</tr>
<tr>
<td>HER-OE</td>
<td>3.08 cm ± 0.203 (N = 93)</td>
<td>4.9%</td>
<td>24.2%</td>
</tr>
<tr>
<td>Triple Negative (TNBC)</td>
<td>3.70 cm ± 0.211 (N = 155)</td>
<td>4.9%</td>
<td>25%</td>
</tr>
</tbody>
</table>

Table 2: Nottingham Prognostic Index components on triple-negative and non-triple-negative breast cancer subtypes

- **Statistics**
  - TNBC vs
  - P-value* = 0.001
  - P-value** < 0.0001
  - P-value*** Not significant

*ANOVA was used to compare the means of the three groups
** P-values were calculated with the use of the χ² test
Which is the definition of Positive SN?

Comments:

Nodal Tumor Burden Is A Continuous (Not Dichotomous) Variable

LN Metastases ≤2mm = Similar Survival as Negative LN (Huvos AG et al 1971).

The ITC Category was introduced In 2003 (AJCC/UICC Staging Manual)

ITC (vs. Micro metastasis): Inter-observer Variability. Uncertain Clinical Significance.

SN results: Predictive (ALND?) and /or Prognostic (DFS, OS)
Which are the optimal technical procedures to assess positivity?

Absolute minimum aim for the histological examination of SNs requires the identification of all macrometastases (an aim that is not currently reached by all laboratories (3)).

Ideally, micrometastases should be identified, because of their estimated association with further nodal involvement (around 20% in general, and possibly over 30% (6) if the micrometastases are > 1 mm).
Which are the optimal technical procedures to assess positivity?
Breast conserving therapy (BCT) is now standard of care in the treatment of patients with invasive breast cancer.

BCT is associated with high levels of local tumor control, but a small proportion of patients develop local recurrence.

Minimizing local recurrence is important because it has impact in the overall survival.
THE GOAL OF MARGIN EVALUATION

• Margins involved is a risk factor for local recurrence.

• Patients with positive margins are *more likely* to have residual disease at or near the primary site than those with negative margins.

• **BUT** a positive margin does not mean residual disease and a negative margin does not preclude extensive residual disease.
THE GOAL OF MARGIN EVALUATION

• IS NOT to ensure that there is no residual tumor in the breast.

• To identify those patients more likely to have a large residual tumor burden and who, therefore, require further surgery.

• To identify those patients unlikely to have a large residual tumor burden and who, therefore, are suitable candidates for BCT without further surgery.
Margins in Surgical Pathology

Colectomy: EASY
Lumpectomy: DIFFICULT!!
HOW TO REPORT MARGIN EVALUATION

• The use of NO INK on tumor as the standard for an adequate margin is associated with low rates of local recurrence and has the potential to decrease re-excision rates, improving cosmetic outcomes, and decreasing health care costs.

• Positive margin= ink on invasive cancer of DCIS.

• Report distance to negative margins in mm.
Molecular Classification of Breast Cancer

**Luminal A:** ER+/PgR+/HER2-

**Luminal B:** ER+/PgR+/HER2+ and/or Ki67+

**HER-OE:** ER-/PgR-/HER2+

**Basal-like:** ER-/PgR-/HER2-/Basal Markers

**Claudin-low:** ER-/Pg-/HER2-/Claudin\textsuperscript{low}
Role of the Pathologist in Breast Cancer

Aim 1: **Diagnosis**
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- Molecular type

Aim 2: **Prognosis**
- Histological type
- Histological grading (plus size and LN status)
- Molecular type

Aim 3: **Prediction**

*Predictive factor is defined as a specific patient or tumor characteristic that correlate with response or lack of response to a specific treatment.*
Breast cancer markers for prediction

- Traditional
  - ER
  - PR
  - HER2
  - Ki67

- Molecular
  - Oncotype Dx 21-gene
  - MammaPrint 70-gene
  - Prosigna
  - Endopredict
ESTROGEN AND PROGESTERONE RECEPTORS

- Immediate impact on systemic treatment
- Weak prognostic markers
- Strong predictive markers of tumor response to hormonal therapy (tamoxifen)
- PR also is independent predictive factor
Recommendations for ER/PR assessment

• If there is a clinical suspicious of breast cancer the specimens should have formalin-fixation.

• If the material was fixed in alcohol then the controls used for the assay must also be fixed in alcohol and need to be validated.

• Antigen-retrieval (heat based) is recommendable.

• Positive and negative controls should be included in every run.

• It is highly desirable to maintain laboratory metrics for each prognostic/predictive test to monitor for potential analytical drift. 70-80% of breast cancer are ER positive.
ER Positive Breast Cancer

Luminal A

Luminal B

60 Sample ER+ Tamoxifen-Treated Test Set
Ma et al., Cancer Cell 5, 1-10 (2004).

45 Tamoxifen Treated Test Set #2
Chang et al., PNAS 102, 3738-43 (2005) + UNC
Meta-Analysis – Gene signatures

Blue dots: good prognosis
Red dots: poor prognosis

Distinction between Luminal A-like and Luminal B-like (HER2 neg) can be:

- Derived from ER, PR and Ki-67? Yes
- If used the minimum value of Ki-67 required for Luminal B-like is:
  - 1-13%: 2.3%
  - 14-19%: 13.6%
  - 20-29%: 36.4%
  - 30% or more: 6.8%
  - Ki-67 should not be used for this distinction: 20.5%
  - Abstain: 2.3%
- Only appropriately determined by multi-gene classifiers: No 66.7%
- Subtype need not be determined since it can be replaced by risk scores derived from multi-gene tests: No 59.5%
Digital image analysis outperforms manual biomarker assessment in breast cancer

Gustav Stålhammar¹,², Nelson Fuentes Martinez¹,³, Michael Lippert⁴, Nicholas P Tobin⁵, Ida Mølholm⁴,⁶, Lorand Kis⁷, Gustaf Rosin¹, Mattias Rantalainen⁸, Lars Pedersen⁴, Jonas Bergh¹,⁵,⁹, Michael Grunkin⁴ and Johan Hartman¹,⁵,⁷

<table>
<thead>
<tr>
<th>Ki67 scoring method</th>
<th>Sensitivity for PAM50 Luminal B vs A</th>
<th>Specificity for PAM50 Luminal B vs A</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIA invasive margin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutoff ≥ 20%</td>
<td>84%</td>
<td>78%</td>
</tr>
<tr>
<td>Cutoff ≥ 20.2% *</td>
<td>82%</td>
<td>79%</td>
</tr>
<tr>
<td>DIA hot spot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutoff ≥ 20%</td>
<td>90%</td>
<td>65%</td>
</tr>
<tr>
<td>Cutoff ≥ 25.2% *</td>
<td>86%</td>
<td>77%</td>
</tr>
<tr>
<td>DIA average</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutoff ≥ 20%</td>
<td>60%</td>
<td>90%</td>
</tr>
<tr>
<td>Cutoff ≥ 15.5% *</td>
<td>80%</td>
<td>83%</td>
</tr>
<tr>
<td>Manual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutoff ≥ 20%</td>
<td>75%</td>
<td>70%</td>
</tr>
<tr>
<td>Cutoff ≥ 22.5% *</td>
<td>74%</td>
<td>75%</td>
</tr>
</tbody>
</table>
Overall Survival Breast Carcinomas
“Pre-trastuzumab”

Ricardo S, JCP 2011

Aparício S, Nature 2012
Invasive breast carcinoma processed with reliable pre-analytical procedures

Score = 0/1+ NEGATIVE

Score = 2+ EQUIVOCAL

Score = 3+ POSITIVE

ISH TESTING

★ NOT AMPLIFIED
★ EQUIVOCAL
★ AMPLIFIED

* HER2 mutation analysis?
- Reflex test
- Careful analysis of ratio and copy numbers
- Look for heterogeneity
- Use an alternative test*
ASCO/CAP HER2 Testing Guideline Update

HER2 testing (invasive component) by validated IHC assay

Batch controls and on-slide controls show appropriate staining

- Circumferential membrane staining that is complete, intense, and within > 10% of tumor cells* → IHC 3+ positive
- Circumferential membrane staining that is incomplete and/or weak/moderate and within > 10% of tumor cells* or Complete and circumferential membrane staining that is intense and within ≤ 10% of tumor cells* → IHC 2+ equivocal
- Incomplete membrane staining that is faint/barely perceptible and within > 10% of tumor cells* → IHC 1+ negative
- No staining is observed* or Membrane staining that is incomplete and is faint/barely perceptible and within ≤ 10% of tumor cells → IHC 0 negative

Must order reflex test (same specimen using ISH) or order a new test (new specimen if available, using IHC or ISH)
HER2 testing (invasive component) by validated dual-probe ISH assay

Batch controls and on-slide controls show appropriate hybridization

HER2/CEP17 ratio ≥ 2.0*

- Average HER2 copy number ≥ 4.0 signals/cell*
  - ISH positive

- Average HER2 copy number < 4.0 signals/cell*
  - ISH positive†

HER2/CEP17 ratio < 2.0

- Average HER2 copy number ≥ 6.0 signals/cell*
  - ISH positive

- Average HER2 copy number ≥ 4.0 and < 6.0 signals/cell*
  - ISH equivocal

- Average HER2 copy number < 4.0 signals/cell
  - ISH negative

Must order a reflex test (same specimen using IHC), test with alternative ISH chromosome 17 probe, or order a new test (new specimen if available, ISH or IHC)
Application of the 2013 ASCO/CAP guideline and the SISH technique for HER2 testing of breast cancer selects more patients for anti-HER2 treatment

António Polónia¹² • Dina Leitão¹² • Fernando Schmitt¹²³

<table>
<thead>
<tr>
<th>HER2 result</th>
<th>Cases before 2013 ASCO/CAP guideline</th>
<th>Cases after 2013 ASCO/CAP guideline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ISH criteria 2007</td>
<td>ISH criteria 2013</td>
</tr>
<tr>
<td>Positive</td>
<td>12.4 % (61)</td>
<td>14.2 % (70)</td>
</tr>
<tr>
<td>Equivocal</td>
<td>3.6 % (18)</td>
<td>1.6 % (8)</td>
</tr>
<tr>
<td>Negative</td>
<td>84.0 % (415)</td>
<td>84.2 % (416)</td>
</tr>
<tr>
<td>Total</td>
<td>494</td>
<td>422</td>
</tr>
</tbody>
</table>
PRE-ANALYTICAL FACTORS
FIXATION TIME

Latéralisation et site de(s) la lésion(s) (si bilatérale, 2 demandes)

☐ Droit
☐ Gauche
Nombre de flacons

Date et heure du prélèvement :
Adequacy of pre-analytical factors

- **Delay of fixation (cold ischemic time)**
  - cold ischemic time: ideal < 1 hour
  - affect morphology: poor tissue preservation, autolysse
  - affect HER2 IHC: negative impact for cases IHC 1+, 2+
  - affect HER2 ISH: loss of HER2 signals and nuclei detail

<table>
<thead>
<tr>
<th>Cold ischemic time</th>
<th>Number of cases uninterpretable (ISH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hour</td>
<td>30%</td>
</tr>
<tr>
<td>&gt; 2 hours</td>
<td>60%</td>
</tr>
<tr>
<td>&gt; 4 hours</td>
<td>80%</td>
</tr>
<tr>
<td>&gt; 6 hours</td>
<td>90%</td>
</tr>
</tbody>
</table>
Adequacy of pre-analytical factors

• Fixative
  – follow the current ASCO/CAP guidelines 2013*
  – 10% neutral buffered formalin (pH 5.0 – 7.0)
  – volume of fixative : sample size = 10:1
  – large samples sliced at 0.5 to 1.0 cm intervals

Adequacy of pre-analytical factors

- **Time of fixation**
  - follow the current ASCO/CAP guidelines 2013*
  - time in fixative: between 6 and 72 hours
  - underfixation: < 6 hours → affect ISH HER2
    - less intensity of signals
    - loss of nuclei structure (overdigestion)

Even driver genetic changes can be heterogeneously distributed in cancers

HER2 immunohistochemistry

HiSeq Exome sequencing + Ion Torrent (4000x) validation
Mutant allele frequencies

Mutant allele freq in HER2− component

Mutant allele freq in HER2+ component

TP53
PIK3CA
ERBB2
ETV5
BRD4
Her2 testing in CNB

<table>
<thead>
<tr>
<th>Reference</th>
<th>Arnedos et al., 2009</th>
<th>Tamaki et al., 2010</th>
<th>D'Alfonso et al., 2010</th>
<th>Apple et al., 2009</th>
<th>Apple et al., 2009</th>
<th>Park et al., 2009</th>
<th>Lebeau et al., 2010</th>
<th>Lee et al., 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples tested (n)</td>
<td>327 353 (patients)</td>
<td>260 260</td>
<td>104 (patients)</td>
<td>500</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testing method</td>
<td>IHC</td>
<td>IHC</td>
<td>FISH</td>
<td>FISH</td>
<td>IHC</td>
<td>IHC</td>
<td>IHC</td>
<td>IHC/FISH</td>
</tr>
<tr>
<td>Overall concordance (%)</td>
<td>98.8</td>
<td>89.3</td>
<td>87</td>
<td>92</td>
<td>98</td>
<td>86.5</td>
<td>90.4</td>
<td>98</td>
</tr>
<tr>
<td>Concordant HER2− [n (%)]</td>
<td>283 (86.5)</td>
<td>182 (96.8)</td>
<td>12 (85.7)</td>
<td>58 (90.6)</td>
<td>83 (66)</td>
<td>—</td>
<td>411 (97.4)</td>
<td>261 (100)</td>
</tr>
<tr>
<td>Concordant HER2+ [n (%)]</td>
<td>40 (12.2)</td>
<td>12 (75.0)</td>
<td>100</td>
<td>13 (10)</td>
<td>6 (5)</td>
<td>—</td>
<td>27 (81.8)</td>
<td>33 (97.0)</td>
</tr>
<tr>
<td>Overall discordance (%)</td>
<td>—</td>
<td>10.66</td>
<td>13</td>
<td>8</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Discordant (CNB+/EX−) [n (%)]</td>
<td>1</td>
<td>0</td>
<td>2 (2)</td>
<td>6 (5)</td>
<td>0 (0)</td>
<td>2</td>
<td>5 (15.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Discordant (CNB−/EX+) [n (%)]</td>
<td>3</td>
<td>0</td>
<td>2 (2)</td>
<td>4 (3)</td>
<td>3 (2)</td>
<td>1</td>
<td>0 (0)</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Indeterminate [n (%)]</td>
<td>4 (1.2)</td>
<td>—</td>
<td>—</td>
<td>0 (0)</td>
<td>33 (26)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

a Scored as IHC 0.
b Scored as IHC 1+.
IHC = immunohistochemistry; FISH = fluorescence in situ hybridization.

<table>
<thead>
<tr>
<th>Rate</th>
<th>HER2 (%) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>93 (80.94–98.5)</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.6 (98.05–100)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>99 (96.97–99.8)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>97.6 (87.14–99.9)</td>
</tr>
</tbody>
</table>
HER2 testing in CNB

Evaluation of HER2 in breast cancer: reality and expectations

Fernanda Milanezi†, Dina Leitão, Sara Ricardo, Isabel Augusto & Fernando Schmitt

†Institute of Molecular Pathology and Immunology of Porto University, Rua Roberto Frias, s/n, 4200-465, Porto, Portugal

• Cases with fixation less than 6 hours and crush artifacts.
• CNB with few tumour cells.
• High grade tumour negative on CNB.
• Low amplified/borderline cases on CNB
Breast cancer markers for prediction

• Probably the most promising and clinically useful area for the application of genetic analysis is the prediction of response to treatment.

• The predictive gene-expression profiles can be used in clinical practice.

• Which patient with ER+ and HER2 negative breast carcinoma will show a good prognosis when treated by endocrine therapy only?
### BREAST CANCER MARKERS FOR PREDICTION

<table>
<thead>
<tr>
<th>Tumor features</th>
<th>Histologic grade</th>
<th>Histologic type</th>
<th>Lymph nodes</th>
<th>Tumor size</th>
<th>ER</th>
<th>HER2</th>
<th>Oncotype DX®</th>
<th>MammaPrint®</th>
<th>Mammostrat®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Features favoring chemo</td>
<td>Grade-3</td>
<td>Ductal (NST)</td>
<td>Positive (&gt; 4)</td>
<td>&gt; 5 cm</td>
<td>ER(-)</td>
<td>(+)</td>
<td>High RS &gt; 30</td>
<td>High risk</td>
<td>High risk index</td>
</tr>
<tr>
<td>Features against chemo</td>
<td>Grade-1</td>
<td>Lobular, tubular, mucinous histology</td>
<td>Negative</td>
<td>&lt; 1 cm</td>
<td>ER(+) High</td>
<td>(-)*</td>
<td>Low RS &lt; 18</td>
<td>Low risk</td>
<td>Low risk index</td>
</tr>
</tbody>
</table>

The decision for adjuvant chemotherapy in a patient ER+/HER2-breast cancer of > 1 cm always requires Ki67 or multi-gene assays?

- **YES:** 55.6%
- **NO:** 44.4%
- **Abstain:** 0

St Gallen Conference 2015
THE ROLE OF PATHOLOGIST IN MOLECULAR TESTING OF BREAST CANCER

Ensure control of preanalytic variables
- Ischemic time
- Fixation time

Selection of tissue for molecular testing

Results reviewed

Discordant results

Correlation of results with histologic and clinical features

Concordant results reported

Reevaluate
- Initial histology/clinical information
- Results/technique of molecular test
- Effect of preanalytic variables
- Consider retesting on additional material
- Discuss with clinician

## Multi-Gene Signatures for Prognosis and Chemotherapy

<table>
<thead>
<tr>
<th>Signature</th>
<th>Prognosis 1-5 y</th>
<th>Prognosis &gt;5 y</th>
<th>Chemo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncotype Dx</td>
<td>82.9% Yes</td>
<td>51.2% No</td>
<td>80.5% Yes</td>
</tr>
<tr>
<td>MammaPrint</td>
<td>89.1% Yes</td>
<td>66.7% No</td>
<td>47.5% No</td>
</tr>
<tr>
<td>PAM-50</td>
<td>92.9% Yes</td>
<td>63.2% Yes</td>
<td>47.1% No</td>
</tr>
<tr>
<td>Endopredict</td>
<td>70.3% Yes</td>
<td>38.2% No</td>
<td>52.9% No</td>
</tr>
</tbody>
</table>
### Comparison of Multiparameter Tests in the UK OPTIMA-Preliminary Trial

Bartlett JMS^1, Stein RC^2, Bayani J^1, Marshall A^1, Dunn JA^3, Campbell AF^1, Cunningham C^4, Sobol M^4, Hall P^5, Rooshenas L^6, Morgan A^7, Poole C^8, Pinder SE^9, Cameron DA^10, Stallard N^11, Donovan J^12, McCabe C^13, Hughes-Davies L^13, Makris A^14 on behalf of the OPTIMA Trial Management Group^15.

<table>
<thead>
<tr>
<th>Number of other tests agreed with test</th>
<th>Oncotype Dx</th>
<th>ROR_PT</th>
<th>MammaPrint</th>
<th>IHC4</th>
<th>IHC4-AQUA</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>119 (39%)</td>
<td>119 (39%)</td>
<td>119 (39%)</td>
<td>119 (39%)</td>
<td>119 (39%)</td>
</tr>
<tr>
<td>3</td>
<td>84 (28%)</td>
<td>77 (26%)</td>
<td>73 (24%)</td>
<td>67 (22%)</td>
<td>75 (25%)</td>
</tr>
<tr>
<td>2</td>
<td>54 (18%)</td>
<td>52 (17%)</td>
<td>47 (16%)</td>
<td>36 (12%)</td>
<td>33 (11%)</td>
</tr>
<tr>
<td>1</td>
<td>31 (10%)</td>
<td>33 (11%)</td>
<td>34 (11%)</td>
<td>25 (8%)</td>
<td>27 (9%)</td>
</tr>
<tr>
<td>0</td>
<td>13 (4%)</td>
<td>18 (6%)</td>
<td>25 (9%)</td>
<td>10 (4%)</td>
<td>17 (6%)</td>
</tr>
<tr>
<td>Missing</td>
<td>1 (1%)</td>
<td>3 (1%)</td>
<td>4 (1%)</td>
<td>45 (15%)</td>
<td>31 (10%)</td>
</tr>
</tbody>
</table>

- Only 31% (n=93) tumours were classified as low/intermediate risk by all five tests.
- 8% (n=26) were classified as high risk by all tests.
- The majority (61%) of cases (n=183) gave no consensus result.
Multigene Molecular Assays in Breast Cancer

Problems

- The results are not based on a homogeneous cohort of patients.
- Does not consider the classical prognostic factors.
- Intermediate score – what to do (Grade II exist here too!)
  (TailorX-tumor size, menopause status)
- Centralized-labs – problems of acceptability for clinicians and pathologists
- Schedule/time to result
Multigene Molecular Assays in Breast Cancer
2nd generation

• Includes the classical prognostic factors combining size and nodal status with gene expression.

• No intermediate group (?).

• Standardized RNA-extraction

• Local certified labs

• Fasts results
PROSIGNA BREAST CANCER PROGNOSTIC GENE SIGNATURE

Block Selected

H&E stain to identify tumor area and cellularity

Tumor area transposed to unstained slides and macrodissected

RNA extracted with manual kit

Extract RNA from FFPE tumor sample

Run RNA and Prosigna CodeSet on nCounter Analysis System

Patient specific expression profile
Is there any role for morphology in therapeutic prediction?
Pathological non-response to chemotherapy in a neoadjuvant setting of breast cancer: an inter-institutional study

Fig. 3 Histograms showing the distribution of response rates for the histotype (a) and the inflammation (b). TILs tumor infiltrating lymphocytes.
Breast Cancer: prognostication and therapy prediction

First Generation Gene Signatures

Systemic Therapy

Second Generation Gene Signatures

Predictive gene signatures

Novel avenues for prognostication and therapy prediction

ER POSITIVE

PROGNOSTIC SIGNATURES

LOW PROLIFERATION

HIGH PROLIFERATION

LOW PROLIFERATION

HIGH PROLIFERATION

HIGHER ENDOCRINE THERAPY BENEFIT

HIGHER CHEMOTHERAPY BENEFIT

Stromal signatures

Predict signature (SET index)

MASSIVE PARALLEL SEQUENCING

ER NEGATIVE

PROGNOSTIC SIGNATURES

HIGH PROLIFERATION

HIGH PROLIFERATION

HIGHER CHEMOTHERAPY BENEFIT

Immune-response signatures

LOW IMMUNE-RESPONSE

HIGH IMMUNE-RESPONSE

LOW BENEFIT OF CHEMOTHERAPY

HIGH BENEFIT OF CHEMOTHERAPY

Predict signatures
Tumor-Associated Lymphocytes As an Independent Predictor of Response to Neoadjuvant Chemotherapy in Breast Cancer

Lymphocyte-predominant breast cancer showed better pathological response to neoadjuvant chemotherapy

intratumoral lymphocytes.
stromal lymphocytes.
breast cancer without a lymphocyte infiltrate is shown

LPBC: lymphocyte-predominant breast cancer tumors
**Morphology**

**Definition and biological relevance**

**Lymphocyte-predominant breast cancer (LPBC)**

Working category to describe tumors with "more lymphocytes than tumor cells". Definitions vary across studies with stromal TILs of 50–60% used as a threshold. LPBC can be used for predefined subgroup analyses and for description of tumors with a particularly high immune infiltrate, however, keep in mind that TILs are a continuous parameter and the threshold for LPBC is still arbitrary.

**Stromal TILs**

Indicator of increased accumulation of immune-cells in tumor tissue

Stromal TILs have been shown to be predictive for increased response to neoadjuvant chemotherapy as well as improved outcome after adjuvant chemotherapy. Based on current data, this parameter is the best parameter for characterization of TILs.

**Intratumoral TILs**

TILs with direct cell-cell contact with carcinoma cells, might be an indicator of direct cell-based anti-tumor effects.

Several studies have shown that intratumoral TILs and more difficult to evaluate and do not provide additional predictive/prognostic information compared to stromal TILs.

**TILs at the invasive margin**

The localization of TILs are the invasive edge is included in the evaluation approach presented in this guideline.

For breast cancer there are no studies with a separate evaluation of TILs at the invasive edge. For practical purposes, the reliable evaluation of the invasive edge might be difficult when using core biopsies in the neoadjuvant setting.

**Tertiary lymphoid structures (TLS)**

Typically localized in the surrounding area of the tumor, TLS might be localized in normal tissue directly adjacent to the tumor, consisting of a T cell zone next to a B cell follicle, often with germinal centers.

While these structures may be important for the biology of tumor-immune reactions, they are not yet optimized for non-research based assessments. The main problem is that TLS have a spatial heterogeneity and are principally localized in areas surrounding the tumor. They might not be in the plane of the tissue section that is being evaluated, in particular when using core biopsies. Furthermore, it might be difficult to distinguish lymphoid aggregates from true TILs, in particular when the germinal center is not in the plane of the section.

**Diagnostic relevance**

**Step 1: Select tumor area**

Include area within tumor borders, do not include immune infiltrate outside of the tumor.

**Step 2: Define stromal area**

Evaluate only TILs in this area - stromal TILs.

**Step 3: Scan at low magnification**

**Step 4: Determine type of inflammatory infiltrate**

Mononuclear stromal TILs infiltrate, do not include granulocytes in necrotic areas.

**Step 5: Assess the percentage of stromal TILs**

Examples of percentages shown in figure 4:

- 0–10% stromal TILs
- 20–40% stromal TILs
- 50–90% stromal TILs

For intermediate group evaluate different areas at higher magnification.
Higher Levels of Tumor-Infiltrating Lymphocytes (TILs) Result in Better Survival Outcomes and Provide Information Independently of Pathological Complete Response (pCR)
Pathologists should include TILs in pathological reports, using the recommended guidelines.

Stromal TILs are the best predictors.

TILs are predictive markers for response in neoadjuvant therapy and prognostic marker in TNBC.

Denkert C, 2015
patients who achieve pathological complete response after neoadjuvant anti-HER2 therapy have longer event-free and overall survival than do patients without pathological complete response.


Patients who attain pathological complete response defined as ypT0 ypN0 or ypT0/is ypN0 have improved survival. The prognostic value is greatest in aggressive tumour subtypes.

Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis

Associations between pathological complete response and EFS and OS
Associations according breast cancer subtype
Pathology of Breast Carcinomas After Neoadjuvant Chemotherapy

An Overview With Recommendations on Specimen Processing and Reporting

Sunati Sahoo, MD; Susan C. Lester, MD, PhD

REPORTING OF BREAST CARCINOMAS AFTER NEOADJUVANT THERAPY

Pathology reports on treated tumors should include the following information.

**Breast Specimen**
1. Presence and size of tumor bed: important for documentation, especially in cases with pathologic complete response.
2. Size and extent of residual tumor
   - Two-dimensional measurements of the largest area of invasive cancer
   - Number of foci or number of blocks with foci of invasion
3. Average cancer cellularity of the residual tumor bed (see Table 1 for Miller-Payne grading system, which requires evaluation of the change in cellularity and for RCB system, which has examples and guidelines to assess residual cellularity)
4. Appearance of the residual tumor and grade, if applicable, compared to pretreatment carcinoma, if possible
5. Viability (necrosis, mitotic figures); proliferation index by MIB-1 (Ki-67) may be requested for some protocols
6. Lymphovascular invasion
7. Presence and extent of ductal carcinoma in situ (percentage of in situ component when using the RCB system)
8. Margins with respect to tumor bed, invasive, and in situ carcinoma
9. A comment on the overall response to treatment

**Lymph Nodes**
1. Number of lymph nodes
2. Number of lymph nodes with metastases
3. Size of the largest metastasis
4. Presence of extranodal extension (measurement of largest extent of extranodal extension may be requested by some radiation oncologists)
5. Number of metastases with evidence of treatment response
6. Number of lymph nodes with evidence of treatment response but without tumor cells (i.e., fibrosis, necrosis, aggregates of histiocytes)

**Classification of Response**
1. By AJCC staging, pT category and pN category assigned a prefix “y” (”p” refers to pathologic classification)
Breast cancer classification

Histopathological types
- Medullary
- Adenoid cystic
- Metaplastic

Subtype defined by IHC markers
- ER-/HER2-
- ER-/HER2+
- ER+/HER2+

Subtypes defined by gene expression
- Claudin low
- Basal like
- HER2 related
- Luminal B
- Luminal A
- Normal like

Structural alterations by NGS

Russnes et al. JCI 2011
The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups

Curtis C et al. Nature
Integrative clusters and survival

The open question:
How can we integrate these subtypes into daily clinical work?
PPP2R2A expression identifies a subgroup of luminal breast cancer with increased risk of relapse and death.
Altered PPP2R2A and Cyclin D1 expression defines a subgroup of aggressive luminal-like breast cancer

Francisco Beca¹,2, Miguel Pereira³, Jorge F Cameselle-Teijeiro⁴, Diana Martins⁵ and Fernando Schmitt⁶*

**Figure 3** Expression levels of PPP2R2A according to CNA. Using data from the TCGA database, the expression levels of PPP2R2A were plotted according to the following classifications of gene copy number: normal (n = 278), heterozygous deletion (HetDel) (n = 411) or homozygous deletion (HomDel) (n = 47). Significant differential expression was observed for all pair-wise comparisons using Student's t-test (p-value <0.001 for all comparisons), showing a positive relationship between copy number and gene expression.

**Figure 4** Survival probability according to CNA on PPP2R2A locus in the TCGA database, carcinomas harboring CNA (either HetDel or HomDel) at the PPP2R2A locus (n = 374) display significantly shorter OS (Wilcoxon p = 0.047).
IHC scoring cut-off level was first determined in discovery cohort.

*Table 1: IHC score cut-off level study in discovery cohort (minimum p-value in Log-Rank test method)*

<table>
<thead>
<tr>
<th>Score</th>
<th>complete cohort</th>
<th>luminal tumors</th>
<th>complete cohort</th>
<th>luminal tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0089</td>
<td>0.0029</td>
<td>45.96 (131)</td>
<td>40.25 (95)</td>
</tr>
<tr>
<td>2</td>
<td>0.0089</td>
<td>0.0029</td>
<td>45.96 (131)</td>
<td>40.25 (95)</td>
</tr>
<tr>
<td>3</td>
<td>0.0024</td>
<td>0.0011</td>
<td>48.77 (139)</td>
<td>41.37 (96)</td>
</tr>
<tr>
<td>4</td>
<td>0.0006</td>
<td>0.0012</td>
<td>68.42 (195)</td>
<td>62.29 (147)</td>
</tr>
</tbody>
</table>
PPP2R2A (B55α) and CCND1 expression status define a ER+ subgroup with worse outcome.

PPP2R2A non expressing carcinomas with CCND1 overexpression
DNA sample
Fragment sample

**Traditional sequencing**
- Clone fragments into bacteria and select clones
  - Grow bacteria, isolate DNA
  - Create multiple copies of single fragment
  - Sequence about 100 fragments in parallel (≈1 Kb each)
  - 0.1 Mb of sequence per "run"

**Next-generation sequencing**
- Separate fragments in fluid or solid substrate
  - With or without PCR amplification
  - Create multiple copies of multiple fragments
  - Sequence millions of fragments in parallel (50 to 100 bp each)
  - >100 Gb of sequence per "run"

---

**Genomic Medicine — An Updated Primer**

W. Gregory Feero, M.D., Ph.D., Alan E. Guttmacher, M.D., and Francis S. Collins, M.D., Ph.D.
Massively Parallel Sequencing-based studies of Breast Cancer

• The collection of genetic aberrations found in breast cancer is complex with a limited number of genes that are frequently mutated in unselected cases.

• The number of genes mutated in small minorities of breast cancer is vast.

• The repertoire of mutations in luminal and basal-like breast cancer is rather different.

• There is no gene or mutation that defines a subtype of breast cancer.

• These studies led to the identification of novel driver genes and that genes that encode ER alpha (ESR1) and HER2 can be targeted by activating mutations.
Emergence of Constitutively Active Estrogen Receptor-α Mutations in Pretreated Advanced Estrogen Receptor–Positive Breast Cancer

A

B

[Diagram illustrating the emergence of mutations in the estrogen receptor-α (ESR1) gene, with Y537C, Y537N, Y537S, D538G, and their genomic alterations labeled.]

[Bar graph showing the alteration percentage of various genes (TP53, PIK3CA, CCND1, MCL1, MYC, FGFR1, ESR1, ERBB2, AKT1, NF1, PTEN) in primary and metastatic cancer samples.]

P < 0.05
It was demonstrated that the majority of HER2 somatic mutations in breast cancer are activating mutations and can be present in non-expressing tumors.

Several patients had mutations that are resistant to lapatinib, but are sensitive to neratinib.

These results suggest that patients with HER2 mutation positive BC could benefit from existing HER2 targeted drugs.
MEASURING CLONAL HETEROGENEITY

Mixture of tumor and normal cells, with mixed clonal composition

Next-generation sequencing separates mixture into single DNA molecules, which are then sequenced

Correction for copy-number variants to obtain clonal-mutation prevalence (not all possible states are represented)

Diploid; expected ratio, 50:50 (A:G)

Single copy gain with loss of heterozygosity; expected ratio, 75:25 (A:G)

Heterozygous deletion; expected ratio, 100:0 (A:G)

Allelic prevalence is estimated from the abundance of aligned sequence reads at each position in the genome
METASTATIC DISEASE? AND NOW
Be sure to treat the present disease

Primary BC
HER-2 negative

Metastases BC
HER-2 positive

Beca F & Schmitt F. Cancer Cytopathology 2014
Table 2. Proportion of Women With a Change in Originally Planned Therapy by Subgroup

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No.</th>
<th>%</th>
<th>Test of Interaction P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>17</td>
<td>14.0</td>
<td></td>
</tr>
<tr>
<td>Lines of therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newly metastatic</td>
<td>7</td>
<td>12.5</td>
<td>.72</td>
</tr>
<tr>
<td>1 prior line of therapy in metastatic setting</td>
<td>2</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>≥2 prior lines of therapy in metastatic setting</td>
<td>8</td>
<td>18.2</td>
<td></td>
</tr>
<tr>
<td>2 lines (n = 14)</td>
<td>2</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>3 lines (n = 8)</td>
<td>1</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>4 lines (n = 4)</td>
<td>1</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>5 lines (n = 4)</td>
<td>1</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>≥6 lines (n = 14)</td>
<td>3</td>
<td>21.4</td>
<td></td>
</tr>
</tbody>
</table>

Duration from primary breast cancer diagnosis and biopsy

<table>
<thead>
<tr>
<th>Quartile</th>
<th>No.</th>
<th>%</th>
<th>Test of Interaction P</th>
</tr>
</thead>
<tbody>
<tr>
<td>First quartile (&lt;35 months)</td>
<td>4</td>
<td>11.4</td>
<td>.15</td>
</tr>
<tr>
<td>Second quartile (36-67 months)</td>
<td>4</td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td>Third quartile (68-118 months)</td>
<td>7</td>
<td>24.1</td>
<td></td>
</tr>
<tr>
<td>Fourth quartile (&gt;118 months)</td>
<td>2</td>
<td>6.5</td>
<td></td>
</tr>
</tbody>
</table>

A

Failure-Free Survival (%) vs. Time to Treatment Failure (months)
# Clinical management of breast cancer heterogeneity

**Dimitrios Zardavas, Alexandre Irrthum, Charles Swanton and Martine Piccart**

<table>
<thead>
<tr>
<th>Type of heterogeneity</th>
<th>Clinical implications</th>
<th>Potential solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intertumour</td>
<td>Need for patient stratification</td>
<td>High-throughput molecular profiling technique Molecular classifiers</td>
</tr>
<tr>
<td></td>
<td>Need for therapy selection/clinical development of targeted agents</td>
<td>Innovative trial designs: Master protocols Basket trials Adaptive trial design N-of-1 studies</td>
</tr>
<tr>
<td>Intratumour</td>
<td>Need to define the phenotype of the recurrent disease</td>
<td>Metastatic biopsy</td>
</tr>
<tr>
<td></td>
<td>Molecular evolution of the disease</td>
<td>Repeated tumour biopsies Geographically separated biopsies Liquid biopsies</td>
</tr>
<tr>
<td></td>
<td>Identification of driver events</td>
<td>Next-generation sequencing Bioinformatic tools and algorithms Systems biology Animal models/functional validation</td>
</tr>
<tr>
<td></td>
<td>Identification of predictive biomarkers</td>
<td>Deep sequencing Single-cell sequencing</td>
</tr>
<tr>
<td></td>
<td>Emergence of treatment resistance</td>
<td>Combination of targeted agents Exploiting passenger events Eradicating the ‘lethal close’ Adaptive therapy Targeting the tumour microenvironment Cancer immunotherapy</td>
</tr>
</tbody>
</table>

For the time being, because of its minimal invasiveness, safety, cost effectiveness, and potential for being coupled to modern ancillary techniques, fine-needle aspiration (FNA) is currently the best method to routinely address the need to repeatedly biopsy the tumor for the purpose of monitoring
PROFILING METASTASES USING FNA

BREAST CANCER, COLON CANCER, LUNG CANCER, MELANOMAS
I am going to finish....
Pathologists Don’t Need to Be Limited to Tissue

*Bright field Dual Color In Situ Hybridization in Circulating Lung Cancer Cells*
Conclusions

• Pathology is a decision-making medical specialty.

• Surgeons and oncologists make decisions based on pathology reports.

• Pathologists are not more “invisible members of the oncology team – we are “diagnostic oncologists”.

IN BREAST CANCER AS DIAGNOSTIC ONCOLOGISTS, PATHOLOGISTS SHOULD BE EXPERTS, GUIDES, LEADERS AND TRANSLATORS SHOWING...

• How molecular testing is currently used to influence clinical practice and treatment decisions in breast cancer.

• How molecular testing has changed our understanding of biology of breast cancer.

• How pathologists can serve as molecular testing consultants and help guide clinical decisions related to these tests.
Breast Cancer Patient Management

Size
Grade
Type
Lymph Node metastasis
Vascular Invasion

“Precision medicine”-based breast cancer patient therapy