RACCOMANDAZIONI DI ST GALLEN 2013

TO FISH OR NOT TO FISH
BREAST CANCER ASSESSMENT: PROGNOSTIC vs PREDICTIVE FACTORS

• PROGNOSTIC FACTORS
correlate with determine outcome
  - May select patients most likely to recur without adjuvant therapy

• PREDICTIVE FACTORS
  reflect the tumor or host response to a specific intervention
  - May select the appropriate therapy for a given individual
BREAST CANCER ASSESSMENT: PROGNOSTIC vs PREDICTIVE FACTORS

1) Age
2) Race
3) Histologic Type
4) Grading
5) pT
6) Node Status
7) Estrogenic Receptors
8) Progestin Receptors
9) Proliferative Index
10) HER2 Status

Gene expression profiling?
While breast cancer is often thought of as a single disease, increasing evidence suggests that there are multiple subtypes of breast cancer:

- occur at different rates in different groups,
- respond to different kinds of treatment,
- are more or less aggressive,
- and have varied long-term survival rates.
- In addition, risk factors may vary for each different subtype of breast cancer.
Stem cells are located in the basal position between the luminal and myoepithelial cells.
Basal cells are a progenitor to the luminal and myoepithelial cells, which are found within the epithelial cell population.
<table>
<thead>
<tr>
<th>Intrinsic Subtype (1)</th>
<th>Clinico-pathologic definition</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>‘<strong>Luminal A</strong>’ ER and/or PgR positive (76) HER2 negative (77) Ki-67 low (&lt;14%)’</td>
<td>This cut-point for Ki-67 labelling index was established by comparison with PAM50 intrinsic subtyping (7). Local quality control of Ki-67 staining is important.</td>
</tr>
<tr>
<td>Luminal B**</td>
<td>‘<strong>Luminal B (HER2 negative)</strong>’ ER and/or PgR positive HER2 negative Ki-67 high</td>
<td>Genes indicative of higher proliferation are markers of poor prognosis in multiple genetic assays (78). If reliable Ki-67 measurement is not available, some alternative assessment of tumor proliferation such as grade may be used to distinguish between ‘Luminal A’ and ‘Luminal B (HER2 negative)’.</td>
</tr>
<tr>
<td></td>
<td>‘<strong>Luminal B (HER2 positive)</strong>’ ER and/or PgR positive Any Ki-67 HER2 over-expressed or amplified</td>
<td>Both endocrine and anti-HER2 therapy may be indicated.</td>
</tr>
<tr>
<td>Erb-B2 overexpression</td>
<td>‘<strong>HER2 positive (non luminal)</strong>’ HER2 over-expressed or amplified ER and PgR absent</td>
<td></td>
</tr>
<tr>
<td>‘Basal-like’</td>
<td>‘<strong>Triple negative (ductal)</strong>’ ER and PgR absent HER2 negative</td>
<td>Approximately 80% overlap between ‘triple negative’ and intrinsic ‘basal-like’ subtype but ‘triple negative’ also includes some special histological types such as (typical) medullary and adenoid cystic carcinoma with low risks of distant recurrence. Staining for basal keratins (79) although shown to aid selection of true basal-like tumors, is considered insufficiently reproducible for general use.</td>
</tr>
</tbody>
</table>
(D) Cumulative breast cancer-specific survival

<table>
<thead>
<tr>
<th>No. events/No. at risk</th>
<th>5-y (95% CI)</th>
<th>10-y (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>151/625</td>
<td>88 (86-91)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>86/263</td>
<td>75 (70-81)</td>
</tr>
<tr>
<td>Luminal/HER2+</td>
<td>20/55</td>
<td>72 (61-85)</td>
</tr>
</tbody>
</table>

Luminal B vs Luminal A log-rank P < .001
Luminal/HER2+ vs Luminal A log-rank P = .02
<table>
<thead>
<tr>
<th>Intrinsic subtype</th>
<th>Clinico-pathologic surrogate definition</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>‘Luminal A-like’</td>
<td>The cut-point between ‘high’ and ‘low’ values for Ki-67 varies between laboratories. A level of &lt;14% best correlated with the gene-expression definition of Luminal A based on the results in a single reference laboratory [23]. Similarly, the added value of PgR in distinguishing between ‘Luminal A-like’ and ‘Luminal B-like’ subtypes derives from the work of Prat et al. which used a PgR cut-point of ≥20% to best correspond to Luminal A subtype [24]. Quality assurance programmes are essential for laboratories reporting these results.</td>
</tr>
<tr>
<td></td>
<td>all of:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ER and PgR positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HER2 negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ki-67 ‘low’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recurrence risk ‘low’ based on</td>
<td></td>
</tr>
<tr>
<td></td>
<td>multi-gene-expression assay (if available)</td>
<td></td>
</tr>
<tr>
<td>Luminal B</td>
<td>‘Luminal B-like (HER2 negative)’</td>
<td>‘Luminal B-like’ disease comprises those luminal cases which lack the characteristics noted above for ‘Luminal A-like’ disease. Thus, either a high Ki-67 value or a low PgR value (see above) may be used to distinguish between ‘Luminal A-like’ and ‘Luminal B-like (HER2 negative)’.</td>
</tr>
<tr>
<td></td>
<td>ER positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HER2 negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>and at least one of:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ki-67 ‘high’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PgR ‘negative or low’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recurrence risk ‘high’ based on</td>
<td></td>
</tr>
<tr>
<td></td>
<td>multi-gene-expression assay (if available)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>‘Luminal B-like (HER2 positive)’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ER positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HER2 over-expressed or amplified</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Any Ki-67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Any PgR</td>
<td></td>
</tr>
<tr>
<td>Erb-B2 overexpression</td>
<td>‘HER2 positive (non-luminal)’</td>
<td>There is an 80% overlap between ‘triple-negative’ and intrinsic ‘basal-like’ subtype. Some cases with low-positive ER staining may cluster with non-luminal subtypes on gene-expression analysis. ‘Triple negative’ also includes some special histological types such as adenoid cystic carcinoma.</td>
</tr>
<tr>
<td></td>
<td>HER2 over-expressed or amplified</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ER and PgR absent</td>
<td></td>
</tr>
<tr>
<td>‘Basal-like’</td>
<td>‘Triple negative (ductal)’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ER and PgR absent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HER2 negative</td>
<td></td>
</tr>
</tbody>
</table>
The Panel noted that standardized cut-offs for Ki67 have not been established and laboratory specific values should be used, but the majority of the Panel (29.5%) voted that a threshold of $\geq 20\%$ was clearly indicative of High Ki67 status.

<table>
<thead>
<tr>
<th>If Ki67 is used which threshold should be used for defining Luminal B subtype?</th>
<th>Yes</th>
<th>No</th>
<th>Abstain</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\geq 14%$?</td>
<td>23.9</td>
<td>37.0</td>
<td>39.0</td>
</tr>
<tr>
<td>$\geq 20%$?</td>
<td>29.5</td>
<td>13.6</td>
<td>56.9</td>
</tr>
<tr>
<td>$\geq 25%$?</td>
<td>13.3</td>
<td>6.7</td>
<td>80.0</td>
</tr>
<tr>
<td>$\geq 30%$? (not voted)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CLASSIFICAZIONE ST. GALLEN 2013
+10% L.A.

CLASSIFICAZIONE ST. GALLEN 2011
CORRELAZIONE SOTTOTIPO-N +

N + CLASSIFICAZIONE 2013 (+11% LA)

- LA: 42%
- LB: 35%
- LBHER: 8%
- HER: 8%
- TN: 7%

N + CLASSIFICAZIONE 2011

- LA: 31%
- LB: 46%
- LBHER: 8%
- HER: 8%
- TN: 7%
<table>
<thead>
<tr>
<th>Subtype</th>
<th>Type of therapy</th>
<th>Notes on therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Luminal A-like’</td>
<td>Endocrine therapy is the most critical intervention and is often used alone.</td>
<td>Cytotoxics may be added in selected patients. Relative indications for the addition of cytotoxics accepted by a majority of the Panel included: (i) high 21-gene RS (i.e. &gt;25), if available; (ii) 70-gene high risk status, if available; (iii) grade 3 disease; (iv) involvement of four or more lymph nodes (a minority required only one node). The Panel was almost equally divided as to whether young age (&lt;35 years) per se was an indication to add cytotoxics. Studies suggest a wide geographical divergence in the threshold indications for the inclusion of cytotoxics for the treatment of patients with luminal disease [96].</td>
</tr>
<tr>
<td>‘Luminal B-like (HER2 negative)’</td>
<td>Endocrine therapy for all patients, cytotoxic therapy for most.</td>
<td>No data are available to support the omission of cytotoxics in this group.</td>
</tr>
<tr>
<td>‘Luminal B-like (HER2 positive)’</td>
<td>Cytotoxics + anti-HER2 + endocrine therapy</td>
<td>Threshold for use of anti-HER2 therapy was defined as pT1b or larger tumour or node-positivity.</td>
</tr>
<tr>
<td>‘HER2 positive (non-luminal)’</td>
<td>Cytotoxics + anti-HER2</td>
<td></td>
</tr>
<tr>
<td>‘Triple negative (ductal)’</td>
<td>Cytotoxics</td>
<td></td>
</tr>
<tr>
<td>‘Special histological types’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Endocrine responsive</td>
<td>Endocrine therapy</td>
<td>Adenoid cystic carcinomas may not require any adjuvant cytotoxics (if node negative).</td>
</tr>
<tr>
<td>B. Endocrine non-responsive</td>
<td>Cytotoxics</td>
<td></td>
</tr>
</tbody>
</table>
Ki67 Index, HER2 Status, and Prognosis of Patients With Luminal B Breast Cancer

Maggie C. U. Cheang, Stephen K. Chia, David Voduc, Dongxia Gao, Samuel Leung, Jacqueline Snider, Mark Watson, Sherri Davies, Philip S. Bernard, Joel S. Parker, Charles M. Perou, Matthew J. Ellis, Torsten O. Nielsen

Background Gene expression profiling of breast cancer has identified two biologically distinct estrogen receptor (ER)-positive subtypes of breast cancer: luminal A and luminal B. Luminal B tumors have higher proliferation and poorer prognosis than luminal A tumors. In this study, we developed a clinically practical immunohistochemistry assay to distinguish luminal B from luminal A tumors and investigated its ability to separate tumors according to breast cancer recurrence-free and disease-specific survival.

Methods Tumors from a cohort of 357 patients with invasive breast carcinomas were subtyped by gene expression profile. Hormone receptor status, HER2 status, and the Ki67 index (percentage of Ki67-positive cancer nuclei) were determined immunohistochemically. Receiver operating characteristic curves were used to determine the Ki67 cut point to distinguish luminal B from luminal A tumors. The prognostic value of the immunohistochemical assignment for breast cancer recurrence-free and disease-specific survival was investigated with an independent tissue microarray series of 4046 breast cancers by use of Kaplan–Meier curves and multivariable Cox regression.

Results Gene expression profiling classified 101 (28%) of the 357 tumors as luminal A and 69 (19%) as luminal B. The best Ki67 index cut point to distinguish luminal B from luminal A tumors was 13.25%. In an independent cohort of 4046 patients with breast cancer, 2847 had hormone receptor–positive tumors. When HER2 immunohistochemistry and the Ki67 index were used to subtype these 2847 tumors, we classified 1530 (59%, 95% confidence interval [CI] = 57% to 61%) as luminal A, 846 (33%, 95% CI = 31% to 34%) as luminal B, and 222 (9%, 95% CI = 7% to 10%) as luminal–HER2 positive. Luminal B and luminal–HER2-positive breast cancers were statistically significantly associated with poor breast cancer recurrence-free and disease-specific survival in all adjuvant systemic treatment categories. Of particular relevance are women who received tamoxifen as their sole adjuvant systemic therapy, among whom the 10-year breast cancer–specific survival was 79% (95% CI = 76% to 83%) for luminal A, 64% (95% CI = 59% to 70%) for luminal B, and 57% (95% CI = 47% to 69%) for luminal–HER2 subtypes.

Conclusion Expression of ER, progesterone receptor, and HER2 proteins and the Ki67 index appear to distinguish luminal A from luminal B breast cancer subtypes.

BREAST CANCER 2012-2013-14
ESPERIENZA OSPEDALE
ONCOLOGICO A. BUSINCO

1131 INVASIVE
BREAST CANCER
SUBTYPES ALL BREAST CANCER

- LA: 40%
- LB: 33%
- LB HER: 8%
- HER: 9%
- TN: 10%
AGE AT PRESENTATION

MEDIA/MEDIANA 58,7

- >70: 23%
- 36-49: 22%
- ≤35: 2%
- 50-69: 53%
AGE AT PRESENTATION

- **LA**
  - >70: 26%
  - 36-49: 20%
  - 50-69: 52%

- **LB**
  - >70: 25%
  - 36-49: 20%
  - 50-69: 53%

- **HER**
  - >70: 12%
  - ≤35: 2%
  - 36-49: 25%
  - 50-69: 61%

- **LBHER**
  - >70: 19%
  - ≤35: 4%
  - 36-49: 28%
  - 50-69: 49%

- **TN**
  - >70: 19%
  - ≤35: 4%
  - 36-49: 27%
  - 50-69: 50%

→ 49 aa 32% LBHER
→ 49 aa 31% TNBC
→ 49 aa 27% HER
GRADING ALL BREAST CANCER

- G1: 12%
- G2: 51%
- G3: 37%
GRADING

LA
- G1: 28% L.A
- G2: 66%
- G3: 6%

LB
- G1: 3%
- G2: 53%
- G3: 44%

HER
- G2: 7%
- G3: 93%

LBHER
- G1: 1%
- G2: 24%
- G3: 75%

TN
- G1: 2%
- G2: 12%
- G3: 86%
NODAL STATUS-ALL BREAST CANCER

- N0: 58%
- N1: 27%
- N2: 9%
- N3: 6%
**NODAL STATUS - SUBTYPES**

- **LA**
  - N0: 66%
  - N1: 26%
  - N2: 6%
  - N3: 2%

- **LB**
  - N0: 52%
  - N1: 30%
  - N2: 17%
  - N3: 16%

- **HER**
  - N0: 56%
  - N1: 28%
  - N2: 7%
  - N3: 9%

- **LBHER**
  - N0: 51%
  - N1: 16%
  - N2: 16%
  - N3: 17%

- **TN**
  - N0: 61%
  - N1: 24%
  - N2: 10%
  - N3: 5%

**Percentage Distribution:***

- **N0**
  - LA: 66%
  - LB: 61%

- **N+**
  - LA: 49% LBHER (33% >4 NODES)
  - LB: 48% LB (30% </3 NODES)
  - HER: 44% HER (16% >4 NODES)
**KI67-SUBTYPES**

- **LA**
  - Ki67 <20: 8%
  - Ki67 20-40: 60%
  - Ki67 >40: 32%
- **LB**
  - Ki67 <20: 15%
  - Ki67 20-40: 85%
- **HER**
  - Ki67 <20: 32%
  - Ki67 20-40: 60%
  - Ki67 >40: 8%
- **LBHER**
  - Ki67 <20: 20%
  - Ki67 20-40: 66%
  - Ki67 >40: 14%
- **TN**
  - Ki67 <20: 12%
  - Ki67 20-40: 65%
  - Ki67 >40: 23%

**Ki67**

- **Ki67 = 20-40%** → 85% LB
- **Ki67 > 40%** → 65% TNBC
  - 32% HER
  - 20% LBHER

- **Ki67 > 40%** → 65% TNBC
HERCEP-TEST

- +++: 13%
- ++-: 11%
- +-+: 16%
- ---: 60%
<table>
<thead>
<tr>
<th>IHC</th>
<th>SISH (11,76%)</th>
<th>AMPLIFICATI (34,6%)</th>
<th>NON AMPLIFICATI (65,4%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>147+++</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>124+++-</td>
<td>106</td>
<td>34 (32%)</td>
<td>72</td>
</tr>
<tr>
<td>181---</td>
<td>15</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>679---</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>TOT.</td>
<td>1131</td>
<td>132</td>
<td>46</td>
</tr>
</tbody>
</table>

POSITIVITA’ HT + SISH: 147 + 34+ 5 +2 = 16,8%
HER2-negative (1+) breast cancer with unfavorable prognostic features: to FISH or not to FISH?

We analyzed 492 invasive breast carcinomas showing HER2 1+ (defined as a faint/barely perceptible membrane staining detected in >10% of the tumor cells) collected from January 2010 to January 2011. FISH was carried out in 84 cases (17%), selected according to one or more of these features: high grade, high Ki-67, extensive vascular invasion, node positivity, and uncertain or absent endocrine responsiveness (Table 1).

In this selected population, 13% of patients with an IHC 1+ breast cancer had a positive FISH result (95% confidence interval 7% to 22%); this result is about two-fold that observed in previous studies (~6.7%).
SISH IN BREAST CANCER WITH UNFAVORABLE PROGNOSTIC FACTORS
ASL8, AOU, AZIENDA BROTZU

• 100 INVASIVE BREAST CANCER IHC ---/+--- WITH UNFAVORABLE PROGNOSTIC FACTORS
LBHER

G1 = 1%  
G2 = 24%  
G3 = 75%

G3 = 93/75% vs 6/44%

LBHER

≤35 4%  
36-49 28%  
50-69 49%  
>70 19%

>50 aa = 27/32% vs 22%  
media 55 aa vs 61/60 aa

LBHER

N0 = 51%  
N1 = 16%  
N2 = 17%  
N3 = 16%

N+ = 44/49% vs 34/48%  
N2-3 = 16/33 vs 8/18%
100 INVASIVE BREAST CANCER

95 NON AMPLIFIED

3 cases ER+, PgR+, AR+, Ki67=20-40%

5 AMPLIFIED

2 cases ER-, PgR-, AR+, Ki67=20-40%
CONCLUSIONS

The key goal of breast cancer diagnosis is to predict the best therapy for patients

1) ST. GALLEN 2013 modified the threshold for Ki67 as indicative of high Ki67 status, mainly because standardized cut-offs for Ki67 have not been established and laboratory specific values should be used. In our dataset, the cut-off proposed has been revealed too much high for defining Luminal A tumors.

2) In our dataset tumors with unfavorable prognostic factors that could be retested in SISH are tumors with:
   a) Luminal phenotype and/or A.R (97%).
   b) Pre-perimenopausal women
   c) G3
   d) N2/N3
   e) Ki67= between 20 and 40%

3) In a very selected dataset the % of HER2 positivity in SISH is 5% of cases

4) It’s not affordable to retest every BC IHCally HER2 negative, but it’s possible to consider to retest a very selected cases to offer the best treatment for patients.
Con la preziosissima collaborazione e l’indispensabile apporto di:

✧ TIZIANA MOI
✧ ELISABETTA SOLLAI
✧ SILVANA URRU

GRAZIE