Molecular Subtyping in Breast Cancer
The Danish Experience

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The Danish Breast Cancer Group

Introduction

- Established in 1977 as a nationwide multidisciplinary organization
- Comprises all departments in Denmark responsible for diagnosis, treatment, follow-up, and research in breast cancer
- Includes a central database
- From 1977 through 2014 ≈ 110,000 women with early invasive breast cancer have been entered into the database
A treatment algorithm is automatically generated by the DBCG database using diagnostic and surgical information entered in real-time from all early breast cancer patients.

### Treatment Algorithm

<table>
<thead>
<tr>
<th>AGE</th>
<th>TUMOR SIZE</th>
<th>NODAL STATUS</th>
<th>HIST and GRADE</th>
<th>ER</th>
<th>HER2</th>
<th>PROG GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥60 år</td>
<td>≤ 10 mm</td>
<td>0</td>
<td>Duktal I, ?, Lobular I-II, ?, Anden type</td>
<td>≥10% / ukendt Medulæer (neg)</td>
<td>Negativ / ukendt</td>
<td>I</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td>≥60 år</td>
<td>&gt; 10 mm</td>
<td>≥ 1</td>
<td>Duktal II-III/Lobular III</td>
<td>0-9%</td>
<td>Positiv</td>
<td>II</td>
</tr>
<tr>
<td>&lt; 60 år</td>
<td>≤ 10 mm</td>
<td>RISIKOFAKTØRER: For pt. ≥40 år med HER2 neg. og ER ≥10% tilbydes KT når der er mindst én risikofaktor, er angivet med &quot;Ja&quot; nedenfor.</td>
<td></td>
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<tr>
<td>RISIKOFAKTØRER: For pt. ≥40 år med HER2 neg. og ER ≥10% tilbydes KT når der er mindst én risikofaktor, er angivet med &quot;Ja&quot; nedenfor.</td>
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</tr>
</tbody>
</table>

### Treatment Flowchart

1. **Prognose Gruppe**
   - **I**
     - **HER2**
       - **Positiv**
         - **0 %**
         - **<40 år**
           - **Ja**: Enge
           - **<40 år**
             - **Ja**: Enge
           - **<40 år**
             - **Ja**: Enge
         - **≥1 %**
         - **40-49 år**
           - **Ja**: KT + ET
           - **<40 år**
             - **Ja**: Enge
         - **≥1 %**
         - **≥50 år**
           - **Præmeno.**
             - **Ja**: Enge
             - **<40 år**
               - **Ja**: Enge
           - **≥50 år**
             - **Postmeno.**
               - **Ja**: Enge
               - **<40 år**
                 - **Ja**: Enge

2. **Treatment**
   - **Beh. ’s-program**
     - 2010 - b
     - 2010 - c
     - 2010 - d
     - 2010 - e

### Risk Factors

- **Risk Factors**
  - **Breast size > 20 mm or node positive or ductal grade 2-3 or lobular grade 3**
  - **Menopausal status**
  - **ER ≥ 10%**

### Website

www.dbcg.dk

Excess mortality derived from the algorithm and divided into quartiles, eg. low risk (Q1), intermediate low (Q2); intermediate high (Q3); and highest risk (Q4). RR with 95% CI is shown for each quartile.
Only one quarter is free of excess mortality when omitting chemotherapy
Q1: Endocrine treatment
Q 2-4: Endocrine treatment and chemotherapy

6529 postmenopausal patients
1996-2004
Treatment algorithm for early breast cancer
Denmark

Treatment allocation

Low Risk: 7% - no systemic treatment
High Risk: 93%
  • 50-60% of the Danish breast cancer patients is allocated to chemotherapy alone or in combination with HER targeted treatment and/or endocrine treatment.

In the process of treatment tailoring we need diagnostic tools (biomarkers) with prognostic information to identify patients who can safely be spared chemotherapy
Gene expression profiling/Prognostic gene signatures

**2000**
- Molecular subtypes
  - Perou et al., Nature 2000
  - Sorlie et al., PNAS 2001

**2002**
- 70-gene signature
  - Amsterdam

**2003**
- 76-gene signature
  - Rotterdam

**2004**
- Oncotype DX
  - 21 genes

**2005**
- Genomic grade Index

**2008...**
- Prosigna: 50 genes, mol subtypes and ROR score
- BCIN: 11 gene assay
- EndoPredict: 12 gene assay

**Molecular subtypes**
- Perou et al., Nature 2000
- Sorlie et al., PNAS 2001

**First gene signature in**
- Low ER+, N- BC (RT-PCR)
- Paik S et al., NEJM 2004

**Important link between proliferation genes and prognostication**
- Sotiriou et al., JNCI 2006
Breast cancer – Molecular intrinsic subtypes

**Endocrine Dependent**
- Favorable Prognosis
- Chemo Resistant

**Endocrine Independent**
- Unfavorable Prognosis
- Chemo Sensitive

**Lum A**
- ER +: 86 % (cut-off ≥ 1%)
- Ki67

**Lum B**
- HER2-
enriched

**HER2 -**
- HER2 +: 13 %

**Double Negative**: app. 10%

**Basal-like**
St Gallen international breast cancer conference on primary therapy of early breast cancer – the road of Ki67

Use of pathology to define intrinsic molecular breast cancer subtypes by application of IHC surrogate markers?

2009 Thresholds for therapies. Ki67: 3 categories low <15%, intermediate 16–30% and high >30

2011 Strategies for breast cancer molecular subtypes genetic testing and attempt for approximation by surrogate IHC markers (ER, PR, HER2 and Ki67) with Ki67 cut off: 14%

2013 Personalizing the treatment of women with early breast cancer. Classification of subtypes with Luminal A: ER+, PR ≥20% and Ki67 <20%, HER2-. Luminal B: ER+ and PR<20% and/or Ki67≥20%, HER2-

2015 Tailoring therapies-improving the management of early breast cancer: Threshold value of Ki-67 within the range of 20%–29% to distinguish ‘luminal B-like` subtype

2017 News since St. Gallen 2015: De-escalating and escalating treatment according to stage and breast cancer subtype: “low” ki67 versus “high” ki67

Assessment of Ki67 in breast cancer. A quality assurance study of the Danish Scientific Committee of Pathology, on behalf of DBCG, in collaboration with the Swedish Breast Pathology Group.

Distribution of observations

Cut-off: >20%

> 20%: 944 (57%)

Lights Kappa:
Inter-rater agreement / multiple raters
Assessment method: 0.664

Cut-off: >20%

>20%: 1113 (67%)

Lights Kappa:
Inter-rater agreement / multiple raters
Count method: 0.649
Ki67: Agreement of observations

<table>
<thead>
<tr>
<th>Assessment Method</th>
<th>Count Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 20%</td>
<td>507</td>
</tr>
<tr>
<td>(72%)</td>
<td>195</td>
</tr>
<tr>
<td>&gt; 20%</td>
<td>26</td>
</tr>
<tr>
<td>(3%)</td>
<td>918</td>
</tr>
<tr>
<td>Total</td>
<td>702</td>
</tr>
<tr>
<td></td>
<td>944</td>
</tr>
<tr>
<td></td>
<td>1646</td>
</tr>
</tbody>
</table>

Observations missing = 6

Proportion of agreement (observations ≤20%): 0.65 – 0.91, median 0.83
Proportion of agreement (observations >20%): 0.73 – 0.96, median 0.87
<table>
<thead>
<tr>
<th></th>
<th>MammaPrint</th>
<th>Oncotype DX</th>
<th>EndoPredict</th>
<th>Prosigna</th>
<th>Breast Cancer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay</strong></td>
<td>Agendia 70-gene assay</td>
<td>Genomic Health 21-gene recurrence score</td>
<td>Myriad (Sividon) 12-gene assay</td>
<td>Nanostring 50-gene assay Mol subtype / ROR score</td>
<td>Biotheranostics 11-gene assay</td>
</tr>
<tr>
<td><strong>Methods</strong></td>
<td>DNA microarray</td>
<td>RT-PCR</td>
<td>RT-PCR</td>
<td>NanoString nCounter</td>
<td>RT-PCR</td>
</tr>
<tr>
<td><strong>Tissue</strong></td>
<td>Frozen or FFPE</td>
<td>FFPE</td>
<td>FFPE</td>
<td>FFPE</td>
<td>FFPE</td>
</tr>
<tr>
<td><strong>Central Analysis</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Population</strong></td>
<td>N0-1</td>
<td>N0-1, ER+</td>
<td>N0-1, ER+/HER2-</td>
<td>N0-1, ER+/HER2-</td>
<td>N0, ER+/HER2-</td>
</tr>
<tr>
<td><strong>Analytical Validity</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Clinical Validity</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Clinical Utility</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>LOE</strong></td>
<td>IA (5 years)</td>
<td>IA (5 years)</td>
<td>IB</td>
<td>IB</td>
<td>IB</td>
</tr>
<tr>
<td><strong>Prospective Trials</strong></td>
<td>MINDACT</td>
<td>TAILORx RxPONDER ADAPT</td>
<td>ADENDOM</td>
<td>OPTIMA PRECISION NEOPAL</td>
<td>Extended Endocrine Treatment</td>
</tr>
</tbody>
</table>
Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline

Lyndsay N. Harris, Nofisat Ismaila, Lisa M. McShane, Fabrice Andre, Deborah E. Collyar, Ana M. Gonzalez-Angulo, Elizabeth H. Hammond, Nicole M. Kuderer, Minetta C. Liu, Robert G. Mannel, Catherine Van Poznak, Robert C. Bast, and Daniel F. Hayes

VOLUME 34 • NUMBER 10 • APRIL 1, 2016
Clinical use of biomarkers in breast cancer: Updated guidelines from the European Group on Tumor Markers (EGTM)

M.J. Duffy a,*, N. Harbeck b, M. Nap c, R. Molina d, A. Nicolini e, E. Senkus f, F. Cardoso g
Of the biomarkers discussed in this article, perhaps the greatest disagreement between different expert panels exists for the measurement of Ki67. Both ASCO [111] and NCCN [22] are opposed to the use of this biomarker because of analytical problems with its measurement and lack of standardisation. In contrast, both ESMO and the St. Gallen Consensus Panel recommend its measurement in specific situations [23,24]. Although analytical problems exist with the current Ki67 assays (see above), because of its wide availability and low cost, the EGTM panel cautiously recommends its measurement, especially in countries in which the more expensive multianalyte tests are not available.
Prediction of 10-year distant recurrence (DR) using the Prosigna® (PAM50) test in a Danish Breast Cancer Cooperative Group (DBCG) cohort of postmenopausal Danish women with hormone receptor-positive (HR+) early breast cancer (EBC) allocated to 5-year of endocrine therapy (ET) alone.

Anne-Vibeke Lænkholm¹, Maj-Britt Jensen², Jens Ole Eriksen¹, Birgitte Bruun Rasmussen³, Ann S. Knoop⁴, Wesley Buckingham⁵, Sean Ferree⁵, Carl Schaper⁵, Torsten O. Nielsen⁶, Taryn Haffner⁷, Torben Kibøl¹, Maj-Lis Møller Talman¹, Anne Marie Bak Jylling⁸, Tomasz Piotr Tabor⁹ and Bent Ejlertsen⁺⁴.

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5. NanoString Technologies, Inc., Seattle, WA, USA
6. Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada
7. Department of Pathology, Rigshospitalet, Copenhagen, Denmark
8. Department of Pathology, Odense University Hospital, Odense, Denmark
9. Department of Pathology, Vejle Hospital, Vejle, Denmark
Excess mortality in postmenopausal high-risk women
Revision of the Prognostic Score Index

<table>
<thead>
<tr>
<th>Standard mortality ratio (SMR)</th>
<th>Q1 (Low risk)</th>
<th>Luminal A Q2</th>
<th>Q3 (Intermediate low risk)</th>
<th>Q4 (Intermediate high risk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>95% CI</td>
<td>0.86-1.06</td>
<td>1.43-1.75</td>
<td>2.21-2.61</td>
<td>5.46-6.29</td>
</tr>
</tbody>
</table>

Excess mortality derived from the algorithm and divided into quartiles, low risk (Q1), intermediate low (Q2); intermediate high (Q3); and highest risk (Q4). RR with 95% CI is shown for each quartile.

Q1: Endocrine treatment
Q 2-4: Endocrine treatment and chemotherapy

In conclusion:

Integration of prognostic gene signature for a selected subgroup of postmenopausal ER+, HER2- patients in national guideline

- Identification of patients who can safely be spared chemotherapy
- DBCG guidelines by April 2017
- Realistic Economy / Cost Effective
- Prespecified risk algorithm including IHC analysis for ER and HER2
- Ki67 is presently not included in the treatment algorithm
Thank you for your attention