Luminal A and B
Where are we?
(or lost in translation?)

Emiel J. Rutgers
How to determine adjuvant or neo-adjuvant treatment for Luminal A or Luminal B cancers?
(as a surgeon)
Disclosures

• No financial interest in a pharmaceutical or diagnostic company
• Not a member of speakers bureau or advisory board
• I’m a surgical oncologist/breast cancer specialist with specific interest in translational research
And some about us

The Netherlands Cancer Institute
The clinical issue

- The prognosis of the patient.
- What is Luminal A?
- What is Luminal B?
- Or how to distinguish Luminal A vs B
- How to treat pts with Luminal A vs Luminal B cancers?
  Should treatment for Luminal A cancers be different from Luminal B cancers

Or….
Is it simpler?
- ER strong +ve & low risk: HT?
- ER +ve plus risk factor: HT + chemotherapy?
Where it all started....
Molecular (‘intrinsic’) subtypes

letters to nature

Molecular portraits of human breast tumours


• Specimens from 65 tumors from 42 patients

Molecular (‘intrinsic’) subtypes

Sørlie et al., PNAS (2003) 100:8418
Immunohistochemistry (‘surrogate subtypes’)
Molecular Subtypes provide insight on which therapies to select (St. Gallen, May 2011)

<table>
<thead>
<tr>
<th>IHC Subtype</th>
<th>Definition</th>
<th>Type of adjuvant therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>HR+/HER2−/Ki67low</td>
<td>Endocrine therapy alone*</td>
</tr>
<tr>
<td>Luminal B</td>
<td>HR+/HER2−/Ki67high</td>
<td>Endocrine therapy ± cytotoxic therapy</td>
</tr>
<tr>
<td>Luminal B</td>
<td>HR+/HER2+</td>
<td>Cytotoxics + anti-HER2 + hormonal therapy</td>
</tr>
<tr>
<td>HER2-positive</td>
<td>HR−/HER2+</td>
<td>Cytotoxics + anti-HER2 therapy</td>
</tr>
<tr>
<td>Triple-negative</td>
<td>HR−/HER2−</td>
<td>Cytotoxic therapy</td>
</tr>
</tbody>
</table>

*A few patients require cytotoxics (such as high nodal status or other indicator of risk). Abbreviation: HR, hormone receptor.*
Classification of breast cancer

Potentials, limitations, challenges

- Morphology:
  - Type
  - Grade
  - pTNM

- Immunohistochemistry:
  - ER
  - PR
  - HER2
    - (Ki-67)

- Signatures:
  - Intrinsic subtypes
  - MammaPrint
  - OncotypeDX
  - TargetPrint
  - BluePrint
The clinical issue.
Think step by step

Step 1: The very low risk cancer: is chemotherapy indicated anyway?
Step 2: Is ER+ve really ER +ve? (or: do you trust your specimen work up system?)
Step 3: If ER +ve is reliably proven, and there is some risk of relapse: adjuvant anti estrogen treatment
Step 4: What are the factors that justify adjuvant chemotherapy (luminal A > B)
The clinical issue. Think step by step

Step 1:
Is prognosis so good that survival advantage of adjuvant chemotherapy outweighs the disadvantages & serious late side effects?
## Clinical determinate cases

<table>
<thead>
<tr>
<th>High Risk</th>
<th>Low Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER negative</td>
<td>ER positive</td>
</tr>
<tr>
<td>Lymph Node positive</td>
<td>Lymph Node negative</td>
</tr>
<tr>
<td>HER2 positive</td>
<td>HER2 negative</td>
</tr>
<tr>
<td>Grade III</td>
<td>Grade I</td>
</tr>
<tr>
<td>Larger tumor size</td>
<td>Small tumor size</td>
</tr>
</tbody>
</table>

Half of our patients are somewhere in between! What to do?
Interobserver agreement morphology and IHC

• Kappa statistics local vs. central assessment
  – Tumor type 0.56
  – Grade 0.50
  – ER 0.85
  – PR 0.72
  – HER2 0.81

Degree of agreement:
0.00-0.20 slight
0.21-0.40 fair
0.41-0.60 moderate
0.61-0.80 substantial
0.81-1.00 (almost) perfect

Is determinate always determinate?

Some examples

• Small cancers good prognosis?
• Grade 1 good prognosis?

➢ There is an important and reproducible discordance between clinical-pathological risk estimates compared to newer techniques by tumor profiling
MammaPrint and Tumorsize T1c BCSS

11 – 22 mm Tumors

Mook et al, Ann Surg Oncol, 2010
DDFS: T1 a/b (n=140)

- Good prognosis, n=85
- Poor prognosis, n=55

HR 3.9 (1.0-15.2)
p=0.05
MammaPrint adds to grading of breast cancer

764 of 1630 patients (47%) were classified as good prognosis and 866 (53%) as poor prognosis by MammaPrint. Histological grading was centrally reviewed for all patients.
DDFS N -ve

Survival Functions

Grade: 1

MammaPrint
0
1
0-censored
1-censored

Cum Survival vs. TIME_DM10
DDFS N-ve

Survival Functions

Grade: 2

Cum Survival

TIME_DM10

MammaPrint
0
1
0-censored
1-censored
### Patient risk allocation

<table>
<thead>
<tr>
<th>Clin-path risk and 70-gene risk at enrollment</th>
<th>Clinical-pathological risk</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>70-gene risk</td>
<td>LOW N(%)</td>
<td>HIGH N(%)</td>
</tr>
<tr>
<td>LOW</td>
<td>2586 (40)</td>
<td>1436 (22)</td>
</tr>
<tr>
<td>HIGH</td>
<td>678 (10)</td>
<td>1827 (28)</td>
</tr>
<tr>
<td>Total</td>
<td>3264 (50)*</td>
<td>3263 (50)*</td>
</tr>
</tbody>
</table>

| Total | 4022 (62) | 2505 (38) |

**Discordant cases (10 + 22 = 32%) match protocol hypothesis**

The **absolute difference** between C-HIGH / G-LOW and C-LOW / G-HIGH is **11.6%**

*The 50-50 split is coincidental*
Oncotype DX and low risk

### B-14 Data NSABP

<table>
<thead>
<tr>
<th>B-14 Data NSABP</th>
<th>Untreated Population</th>
<th>Treated Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast Cancer Mortality</td>
<td>(95%CI, 355 pat)</td>
<td>(85%CI) (290)</td>
</tr>
<tr>
<td><strong>Low Risk</strong> (RS&lt;18) (313 pat)</td>
<td><strong>14.1%</strong> (19.5%, 8.64%) (171 pat)</td>
<td><strong>6.9%</strong> (11.2%, 2.5%) (142 pat)</td>
</tr>
<tr>
<td>Int Risk (RS 18-30) (154 pat)</td>
<td>37.8% (48.9%, 26.8%) (85 pat)</td>
<td>20.5% (30.4%, 10.5%) (69 pat)</td>
</tr>
<tr>
<td>High Risk (RS≥31) (178 pat)</td>
<td>31.3% (40.9%, 21.8%) (99 pat)</td>
<td>29.7% (40.2%, 19.3%) (79 pat)</td>
</tr>
</tbody>
</table>
70-Gene signature (MammaPrint) prospectively predicts prognosis of patients with node-negative breast cancer: 5 year follow-up of the RASTER study

5-year distant recurrence-free interval

MammaPrint

- Low risk: 97%
- High risk: 91.7%
- p=0.03

AOL

- Low risk: 96.7%
- High risk: 93.4%
- p=NS
MammaPrint in observational prospective trial
RASTER study, 5-year DDFS of 427 patients according to 70GPS or AOL

5YR DDFS

70-gene signature

208 (49%)

219 (51%)

89.8%

96.1%

Adjuvant Online

295 (69%)

132 (31%)

70 pts no AST:
DDFS 100%

5YR DDFS

92.4%

94.4%

Linn, Rutgers, Drukker, et al., EBCC 2012
Discordant cases who received no AST

60 mos mLow-cLow 95.0% (95%CI 90.3-99.9)
60 mos mLow-cHigh 100% (95%CI 100-100)
Discordant cases who received no AST or endocrine therapy only

- 60 mos mLow-cLow 94.1% (95%CI 89.1-99.3)
- 60 mos mLow-cHigh 97.8% (95%CI 94.9-100)

n=94  
n=92
Role of Ki-67
RFS luminal A vs. B based on Ki-67

Cheang et al., JNCI (2009) 101: 736-750
Assessment of Ki67 in Breast Cancer: Recommendations from the International Ki67 in Breast Cancer Working Group

Mitch Dowsett, Torsten O. Nielsen, Roger A’Hern, John Bartlett, R. Charles Coombes, Jack Cuzick, Matthew Ellis, N. Lynn Henry, Judith C. Hugh, Tracy Lively, Lisa McShane, Soon Paik, Frederique Penault-Llorca, Ljudmila Prudkin, Meredith Regan, Janine Salter, Christos Sotiriou, Ian E. Smith, Giuseppe Viale, Jo Anne Zujewski, Daniel F. Hayes

Manuscript received March 14, 2011; revised September 1, 2011; accepted September 2, 2011.
Mitch Dowsett (Mr. Ki-67):

• Ki-67 may identify luminal class with a cut-off level of 13.25% proposed to distinguish poorer prognosis luminal B cancers from luminal A
  – Lack of between laboratory standards limiting application as a surrogate marker
• Standardized methodologies for Ki-67 are lacking
  – ASCO Tumor Marker Guidelines Committee: clinical utility of Ki-67 insufficient to recommend routine use for prognostic purposes
  – In 2011, the International Ki-67 in Breast Cancer Working Group published recommendations for Ki-67 assessment in breast cancer

• Cut points for prognosis, prediction, and monitoring should only be applied if the results from local practice have been validated against those in studies that have defined the cutoff for the intended use of the Ki67 result.

Box 1. Recommendations for Ki67 assessment in breast cancer

Preanalytical
• Core-cut biopsies and whole sections from excision biopsies are acceptable specimens; when comparative scores are to be made, it is preferable to use the same type for both samples (e.g., in presurgical studies).
• TMA s are acceptable for clinical trial evaluation or epidemiological studies of Ki67.
• Fixation in neutral buffered formalin should follow the same guidelines as published for steroid receptors (39,40).
• Once prepared, tissue sections should not be stored at room temperature for longer than 14 days. Results after longer storage must be viewed with caution.

Analytical
• Known positive and negative controls should be included in all batches; positive nuclei of nonmalignant cells and with mitotic figures provide evidence of the quality of an individual section.
• Antigen retrieval procedures are required. The best evidence supports the use of heat-induced retrieval most frequently by microwave processing.
• The MIB1 antibody is currently endorsed for Ki67.

Interpretation and scoring
• In full sections, at least three high-power (×40 objective) fields should be selected to represent the spectrum of staining seen on initial overview of the whole section.
• For the purpose of prognostic evaluation, the invasive edge of the tumor should be scored.
• If pharmacodynamic comparisons must be between core cuts and sections from the excision, assessment of the latter should be across the whole tumor.
• If there are clear hot spots, data from these should be included in the overall score.
• Only nuclear staining is considered positive. Staining intensity is not relevant.
• Scoring should involve the counting of at least 500 malignant invasive cells (and preferably at least 1000 cells) unless a protocol clearly states reasons for fewer being acceptable.
• Image analysis methods for Ki67 remain to be proven for use in clinical practice.

Data handling
• The Ki67 score or index should be expressed as the percentage of positively staining cells among the total number of invasive cells in the area scored.
• Statistical analysis should take account of the log-normal distribution generally followed by Ki67 measurement.
• The most appropriate endpoint in comparative studies of treatment efficacy or response is the percentage suppression of Ki67-positive cells.
• The most appropriate endpoint for assessing residual risk of recurrence is the on-treatment proportion of Ki67-positive cells.
• Cut points for prognosis, prediction, and monitoring should only be applied if the results from local practice have been validated against those in studies that have defined the cutoff for the intended use of the Ki67 result.
The clinical issue.

Think step by step

Step 1:

Is prognosis so good that survival advantage of adjuvant chemotherapy outweighs the disadvantages & serious late side effects?

Can we select those patients?

My conclusion:

- on the basis of standard clinical-pathological data only a few. Ki-67 is of limited help, only in the extremes
- You need to add extra information on the molecular tumor biology of the primary to be able to select a larger proportion (40% vs 10% of early node negative ER +ve breast cancers)
The clinical issue.
Think step by step

Step 2:
  Is ER +ve really ER +ve?

Or:
- How reliable is your ER IHC scoring?
- Where is the cut-off?
Stages of IHC testing

- **Pre-analytic**
  - Transport
  - Secretary support
  - Tissue, type, and dimension
  - Decalcification
  - Preparation
  - Fixation
    - Time, Type, Volume
  - Section
    - Thickness
    - Storage & Drying

- **Analytic**
  - Antigen retrieval
  - Primary antibody
    - Clone
    - Dilution
    - Buffer
    - Time
    - Temperature
  - Manual vs. Automated
  - Development
  - Visualization
  - Sensitivity
  - Specificity

- **Post-analytic**
  - Interpretation
  - Localization
  - Cut-off
  - Quantification
  - Reporting
  - Secretary support!
  - Control
    - Internal
    - External
  - Quality assessment
The perfect test is non-existent

- No 100% sensitivity
- No 100% specificity
ER-status based on IHC, mRNA, and signature (n=456 FNAs)

**mRNA**

**signature**

Fig 1. Estrogen receptor (ER) mRNA and ER-associated gene expression in four distinct immunohistochemistry groups. Immunohistochemistry groups were defined by the percentage of cells that were positive for nuclear ER staining. (A) Expression distribution of ESR1 mRNA. (B) ER-associated gene signature refers to the average expression of 108 probe sets that are highly coexpressed with ESR1. P values were calculated with the Wilcoxon test.
IHC and mRNA ER-status and OS

DFS and ER-status in BIG 1-98 trial

High concordance of protein (by IHC), gene (by FISH; HER2 only) and microarray readout (by TargetPrint) of ER/PR/HER2: results from the MINDACT trial

B. Viale et al. (2011) ASCO

Background
This study was undertaken to further determine the concordance of microarray readout by TargetPrint with IHC/FISH assessment both locally and centrally determined in the first 800 pts enrolled in the MINDACT trial (Rutgers et al., 2007, JCO).

This work was essential to determine the quality of biological assays in the two risk assessment method used in MINDACT based upon which adjuvant chemotherapy decision is made, in order to exclude bias.

Results
Local pathology assessment with central review

Comparisons of local assessment (IHC & FISH for HER2) with central review (n=248) indicated highly similar results for protein readout with a concordance of 92% (k=0.86) for ER, and 93% for HER2 (k=0.80) and slightly lower for PR (90% k=0.72).

Central review with microarray readout by TargetPrint

Comparison of central review (n=248) with microarray readout by TargetPrint indicated highly similar results for receptor readout with a concordance of 96% (k=0.85) for ER; 95% for HER2 (k=0.79) and lower for PR (87%) (k=0.63).

Variability

Inter-observer variability for ER, PR and HER2 has been reported and has informed standardisation protocols. Indicating the need for a stable and reliable result for these prognostic parameters.

To be considered acceptable, the results of the assay must be initially 90% concordant with those of the clinically validated assay for the ER- and PR-positive category and 93% concordant for the DR- or PR-negative category. For HER2, concordance in the positive category is important.

Conclusion
Locally and centrally assessed ER, PR and HER2 status in the first 800 pts (626 centrally assessed) MINDACT patient samples indicate a high quality level of pathology in the local participating hospitals. These results exclude any bias induced by a lower quality of traditional pathology results as compared to the centrally assessed MammaPrint, both used for risk assessment and adjuvant chemotherapy decision in the MINDACT trial. The microarray-based assessment of ER, PR and HER2 gives results comparable to IHC & FISH and provides an objective and quantitative assessment of tumor receptor status. These results indicate that TargetPrint can serve as a second pathology assessment for locally assessed parameters, especially since TargetPrint is part of a multi-profile platform for breast cancer treatment management.
Results

Central review with microarray readout by TargetPrint

Comparison of central review (n=626) with microarray readout by TargetPrint indicated highly similar results for receptor readout with a concordance of 98% (k=0.90) for ER; and 96% for HER2 (k=0.78) and lower for PR (85% (k=0.62)).

<table>
<thead>
<tr>
<th></th>
<th>central ER</th>
<th></th>
<th>central PR</th>
<th></th>
<th>central HER2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pos 525</td>
<td>neg 12</td>
<td>pos 408</td>
<td>neg 82</td>
<td>pos 47</td>
<td>neg 14</td>
</tr>
<tr>
<td>n=619*</td>
<td></td>
<td></td>
<td>n=619*</td>
<td></td>
<td>n=614*</td>
<td></td>
</tr>
<tr>
<td>pos</td>
<td>3</td>
<td></td>
<td>11</td>
<td></td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>neg</td>
<td>79</td>
<td></td>
<td>118</td>
<td></td>
<td>7</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Estrogen receptor

Progesterone receptor

HER2 receptor
High concordance for microarray based determination of ER, PR and HER2 receptor status and local IHC/FISH assessment worldwide in 827 patients

J. Wesseling et al. (2011) ASCO

Background
- The level of estrogen receptor (ER), progesterone receptor (PR) and HER2 expression is predictive for prognosis and/or treatment response in breast cancer patients.
- Differences in fixation and subjective interpretation can substantially affect the accuracy and reproducibility of the results in IHC.
- The commercially available TargetPrint test measures the mRNA expression level of ER, PR and HER2 and provides an objective and standardized alternative to IHC.

Methods
- Tumor samples (n=831) from breast cancer patients (stage I to IV) were collected prospectively worldwide between 2008 and 2011 by core needle biopsy or from a surgical specimen.
- The mRNA level of ER, PR and HER2 was assessed with TargetPrint.
- IHC/FISH assessments were performed according to local standards at the participating hospitals.
- HER2 IHC scores of 0 or 1 were considered negative. An IHC score of 3+ was considered positive. IHC 2+ cases with an amplified FISH result were considered positive and non-amplified FISH results negative.
- HER2 IHC/FISH was unknown for 12 samples; ER/PR IHC unknown for 4.
- IHC staining results were compared to the quantitative gene expression results (TargetPrint).
- Discordant cases were centrally reviewed for IHC/FISH assessment.

IHC versus TargetPrint (microarray)

Results central review of discordant cases
- Central re-assessment (blind for original results) for IHC ER/TargetPrint ER cases indicated TargetPrint to be used as second opinion in such cases.
- For HER2, microarray readout shows true discordance for a number of discordant cases. Further research is indicated.

Concordance & kappa statistics for ER, PR, and HER2

Overall comparison of IHC and gene expression (mRNA level) read out by TargetPrint shows a concordance of
95% for ER; 83% for PR and 94% for HER2

Institutional data: the concordance between centers ranged from 88-100% for ER, 77-95% for PR, and 91-100% for HER2*

* Ranges were calculated from institutes with more than 20 cases

Percent agreement for ER, PR, and HER2

Conclusion
- Microarray based readout of ER, PR and HER2 status using TargetPrint is highly comparable to local IHC and FISH analysis in 827 analyzed samples worldwide.
- The results indicate mRNA expression read out for ER, PR and HER2 by TargetPrint provides high quality second opinion for local IHC/FISH assessment.
- First central re-assessment of 103 discordant assessments are shown and discussed.

For further information on the comparison of local IHC read out and TargetPrint please visit: P3-04-06 and P1-07-06

J. Wesseling et al. (2011) ASCO
Reliable ‘second opinion’

<table>
<thead>
<tr>
<th></th>
<th>Concordance (95% CI)</th>
<th>Kappa (95% CI)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>98% (96-99%)</td>
<td>90% (85-95%)</td>
<td>619</td>
</tr>
<tr>
<td>PR</td>
<td>85% (82-88%)</td>
<td>62% (55-69%)</td>
<td>619</td>
</tr>
<tr>
<td>HER2</td>
<td>96% (94-97%)</td>
<td>78% (70-86%)</td>
<td>614</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>ER</th>
<th>PR</th>
<th>HER2</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive agreement</td>
<td>525/537 = 98%</td>
<td>408/490 = 83%</td>
<td>53/74 = 72%</td>
</tr>
<tr>
<td>negative agreement</td>
<td>79/82 = 96%</td>
<td>118/129 = 91%</td>
<td>535/540 = 99%</td>
</tr>
<tr>
<td>NPV/PPV</td>
<td>NPV = 87%</td>
<td>NPV = 59%</td>
<td>PPV = 91%</td>
</tr>
</tbody>
</table>
The clinical issue. Think step by step

Step 2:
Is ER +ve really ER +ve?

My conclusion (for debate):
• Have your ER testing done by standard operational procedures, including quality control programs in sufficient case load labs.
• IHC is good
• Threshold: like ASCO-CAP guidelines: >1% consider anti ER therapy
The clinical issue.
Think step by step (the most easy one)

Step 3:
If ER +ve is reliably proven, and there is some risk of relapse

- Adjuvant anti-estrogen treatments: effect is proven
  - At least 5 years
  - Premenopausal: tamoxifen +/- ovarion ablation
  - Postmenopausal at least 2-3 years AI (+ tamoxifen) or AI only
  - In higher risk: extended to 7 (10?) years
Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials

Early Breast Cancer Trialists' Collaborative Group (EBCTCG)

Summary
Background: As trials of 5 years of tamoxifen in early breast cancer mature, the relevance of hormone receptor

Figure 1: Relevance of measured ER and PR status to the effects of about 5 years of tamoxifen on the 10-year probability of recurrence.

Greatest (beneficial) effect of tamoxifen is seen in patients with ER+ve tumors (Table 1).

Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials

Background. As trials of 5 years of tamoxifen in early breast cancer mature, the relevance of hormone receptor

Summary

<table>
<thead>
<tr>
<th>Category</th>
<th>Event rate (yes per 1,000)</th>
<th>Tamoxifen events</th>
<th>Rate ratio (95% CI)</th>
<th>Death rate (yes per 1,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-0</td>
<td>167 (10.8)</td>
<td>161 (10.6)</td>
<td>0.95 (0.81 to 1.12)</td>
<td>1.2 (0.9 to 1.6)</td>
</tr>
<tr>
<td>PR-0</td>
<td>167 (10.8)</td>
<td>161 (10.6)</td>
<td>0.95 (0.81 to 1.12)</td>
<td>1.2 (0.9 to 1.6)</td>
</tr>
<tr>
<td>ER-PR-</td>
<td>167 (10.8)</td>
<td>161 (10.6)</td>
<td>0.95 (0.81 to 1.12)</td>
<td>1.2 (0.9 to 1.6)</td>
</tr>
<tr>
<td>ER-PR+</td>
<td>167 (10.8)</td>
<td>161 (10.6)</td>
<td>0.95 (0.81 to 1.12)</td>
<td>1.2 (0.9 to 1.6)</td>
</tr>
<tr>
<td>PR-ER-</td>
<td>167 (10.8)</td>
<td>161 (10.6)</td>
<td>0.95 (0.81 to 1.12)</td>
<td>1.2 (0.9 to 1.6)</td>
</tr>
<tr>
<td>PR-ER+</td>
<td>167 (10.8)</td>
<td>161 (10.6)</td>
<td>0.95 (0.81 to 1.12)</td>
<td>1.2 (0.9 to 1.6)</td>
</tr>
<tr>
<td>ER-unknown</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR-unknown</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (ER-0+PR-0)</td>
<td>347 (23.0)</td>
<td>341 (22.6)</td>
<td>0.99 (0.86 to 1.14)</td>
<td>1.1 (0.8 to 1.5)</td>
</tr>
<tr>
<td>Total (ER-PR)</td>
<td>347 (23.0)</td>
<td>341 (22.6)</td>
<td>0.99 (0.86 to 1.14)</td>
<td>1.1 (0.8 to 1.5)</td>
</tr>
</tbody>
</table>

Figure 2: Relevance of quantitative ER and PR measurement (femtograms per milliliter) to the tamoxifen versus control recurrence rate ratio. Outcome by allocated treatment in trials of about 5 years of adjuvant tamoxifen. Other ER power includes ER-negative by immunohistochemistry and ER unspecified, but less than 10 femtograms. ER-estrogen receptor, PR-progesterone receptor. 0-4 observed minus expected.

Difference between treatment effects in subtotals (a-d) and (i-l) is statistically significant (p < 0.001).
Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials

*Early breast cancer trials Collaborative Group (EBCTCG)*

**Summary**

Background: As trials of 5 years of tamoxifen in early breast cancer mature, the relevance of hormone receptor

![Graphs showing the effect of adjuvant tamoxifen](image)

**Figure 2:** Relevance of nodal status and of background chemotherapy to the effects of tamoxifen on the 10-year probability of recurrence, for ER-positive disease. Outcomes by allocated treatment in trials of about 5 years of adjuvant tamoxifen. Event rate ratio (ER) is from weighted log-rank statistics for all time periods. Gain (and its SE) is absolute difference between ends of graphs. ER-negative receptor. ER-positive receptor. ER-untested when untested, with variance only.
Step 3:
If ER +ve is reliably proven, and there is some risk of relapse

My conclusions:
- Adjuvant anti-estrogen treatments: effect is proven
  - At least 5 years
  - Premenopausal: tamoxifen +/- ovarion ablation (role await SOFT trial results)
  - Postmenopausal at least 2-3 years AI (+ tamoxifen) or AI only
  - In higher risk: extended to 7 (10?) years: see upcoming ATLAS trial results!
The clinical issue.
Think step by step
(the most difficult one)

Step 4: What are the factors that justify adjuvant chemotherapy (luminal A > B)

- Is every luminal A a luminal A?
- What makes luminal B a luminal B?
- What is the effect of chemotherapy: different for luminal A or B?
What are Intrinsic Molecular Subtypes?

• Molecular subtypes show which pathway drives cancer growth.
  – Luminal it is the estrogen pathway
  – ERB2 it is the HER2 pathway
  – Basal it is neither one of them

• There is approx 20% discordance between molecular subtypes and subtyping with IHC (Perou 2011)

Red = Up-regulation
Green = Down-regulation
Is molecular subtyping useful in “fine tuning” your treatment decisions?

First some supportive data…
Response to neo-adjuvant chemotherapy in molecular subgroups

<table>
<thead>
<tr>
<th>Molecular Subtype</th>
<th>Straver (1)</th>
<th>Somlo (2)</th>
<th>Krijgsman (3)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Risk Luminal-type</td>
<td>n: 21</td>
<td>n: 14</td>
<td>n: 29</td>
<td>n: 64</td>
</tr>
<tr>
<td>HER2-type</td>
<td>n: 41</td>
<td>n: 18</td>
<td>n: 24</td>
<td>n: 83</td>
</tr>
</tbody>
</table>

A diagnostic gene profile for molecular subtyping of breast cancer associated with treatment response

Oscar Krijgsman · Paul Roepman · Wilbert Zwart · Jason S. Carroll · Sun Tian · Femke A. de Snoo · Richard A. Bender · Rene Bernards · Annuska M. Glas
A diagnostic gene profile for molecular subtyping of breast cancer associated with treatment response

Oscar Krijgsman · Paul Roepman · Wilbert Zwart · Jason S. Carroll · Sun Tian · Femke A. de Snoo · Richard A. Bender · Rene Bernards · Annuska M. Glas

- PcR and 5yr follow-up of neoadjuvant patients confirms the very response to chemotherapy of Luminal Low Risk patients.
- PcR rates confirm that there is a benefit of chemotherapy in Luminal High Risk patients.
- PcR rates in Basal & HER2 are high stressing the importance of identifying the subtype in these two groups.
Molecular Subtyping Signature
80-gene signature
Profiles Basal, Luminal and HER2 subtypes
Response and long term outcomes after neo-adjuvant chemotherapy: Pooled dataset of patients stratified by molecular subtyping by MammaPrint and BluePrint

Stefan Glück1, Femke de Snoo2, Justine Peeters2, George Somlo3, Laura van’t Veer4
1. University of Antwerp, Antwerp Comprehensive Cancer Center, Belgium, 2. Agenzia, Amsterdam, Netherlands, 3. City of Hope, Duarte, CA, 4. UCM, San Francisco, CA

**Background**
Classification of breast cancers into molecular subtypes may be important for the appropriate selection of therapy for patients with early breast cancer. Previous analyses had shown that breast cancer subtypes have distinct clinical outcomes (Soulie, PNAS, 2001; Esserman, BCRT, 2011). In our study, we analyze using MammaPrint together with an 80-gene molecular subtyping profile (BluePrint) the response to neo-adjuvant chemotherapy and long term outcomes.

**Methods**
This study was carried out on data from 421 patients: 141 patients from the I-SPY 1 trial; 230 patients from biomarker discovery program at MD Anderson (131 and 99 respectively; Hess et al., 2006, JCO; Iwamoto et al., 2011, BCRT); and 50 patients from City of Hope (Somlo et al., ASCO, 2010). All patients were treated in the neo-adjuvant setting with chemotherapy. MammaPrint and BluePrint outcomes were determined from either 44K Agilent arrays run at Agendia or available through the I-SPY 1 data portal, or from Affymetrix U133A arrays. The combination of MammaPrint and BluePrint resulted in 4 distinct molecular groups: Luminal A (MammaPrint Low Risk/Luminal-type), Luminal B (MammaPrint High Risk/Luminal-type), Basal-type and HER2-type.

**Addition value of Molecular Subtyping**
Luminal A patients (BluePrint Luminal/ MammaPrint Low Risk) have a good baseline prognosis with excellent survival and may have no benefit from chemotherapy.

A subset of clinical HER2+ patients are classified as Luminal-type by BluePrint. The BluePrint HER2-type pCR patients have a 5 yr DMFS of 87%, compared to clinical HER2+ pCR patients who have 78% 5 yr DMFS. A recent pooled analysis showed that pCR rate is low in clinical HER2+/Luminal patients and is not associated with outcome (von Minckwitz et al., 2012, JCO). BluePrint classifies more patients as Basal-type (n=120) with higher pCR rate (42%), compared to clinical subtyping (n=93) with a pCR rate of 31%.

**Summary**
Molecular Subtyping can improve stratification of patients in the neo-adjuvant setting; MammaPrint Low Risk patients have a good baseline prognosis with excellent survival and may not benefit from chemotherapy. We observed marked differences in response and DMFS to neo-adjuvant treatment in groups stratified by MammaPrint and BluePrint. These findings confirm differences in chemotherapy response among molecular subgroups, and indicate that BluePrint and MammaPrint help to further establish a clinical correlation between molecular subtyping and treatment outcomes.
Key Findings:

- 5 year survival data suggests that a combination of MammaPrint and BluePrint more accurately identifies Luminal, Basal and ERB2 subtypes compared to IHC.
Key Findings:

- 42% of patients that were classified as HER 2+ by IHC/ Fish were reclassified as Luminal’s with Blue Print
- Luminal A’s have a 5yr DMFS of 94%.
- If significant co-morbidities exist is it worth considering withholding Herceptin from Luminal A patients?
Is molecular subtyping useful in “fine tuning” your treatment decisions?

Than some sobering data…. 
Concordance single sample predictors (SSP)

A NKI-295 dataset

Sorlie SSP

- Basal-like
- HER2
- Luminal A
- Luminal B
- Normal breast-like

Weigelt et al., Lancet Oncology (2010) 11: 229
Concordance SSP algorithms

A  NKI-295 dataset
- Sorlie SSP
- Hu SSP
- Parker SSP
- ER status
- Histological grade
- Lymph-node status
- Tumour size

B  Sorlie SSP 2003
- Overall survival (%)
- Patients at risk: Basal-like 47, HER2 46, Luminal A 96, Luminal B 77, Normal breast-like 29

C  Hu SSP 2006
- Overall survival (%)
- Patients at risk: 56, 37, 133, 48, 21

D  Parker SSP 2009
- Overall survival (%)
- Patients at risk: 48, 54, 90, 78, 25

Weigelt et al., Lancet Oncology (2010) 11: 229
Concordance molecular vs. IHC subtyping (n=560)

Lips et al., submitted
Comparison of molecular (BluePrint+MammaPrint) and pathological subtypes for breast cancer among the first 800 patients from the EORTC 10041/BIG 3-04 (MINDACT) trial

Giuseppe Viale1, Leen Slaets2, Femke de Snoo3, Laura J. van ‘t Veer4, Emil J. Rutgers5, Martine Piccart6, Jan Bogers7, Jeroen van den Akker3, Kristel Engelen2, Leila Russo3, Patrizia Dell’Olio7, Fatima Cardoso7

1. European Institute of Oncology and University of Milan, Italy; 2. European Organisation for Research and Treatment of Cancer, Brussels, Belgium; 3. Agendia, Amsterdam, Netherlands; 4. UCL, San Francisco, US; 5. Netherlands Cancer Institute, Amsterdam, Netherlands; 6. John Berken Institute, Brussels, Belgium; 7. Breast Unit, Champalimaud Cancer Center, Lisbon, Portugal

Background
Biology has become the main driver of breast cancer therapy. Intrinsic biological subtypes by gene expression profiling have been identified. Pathology can be used to define surrogates of these subtypes but these are not always concordant, which may lead to different treatment plans. We investigated the concordance between BluePrint + MammaPrint (micro array based) breast cancer subtypes and pathological surrogates (based on ER, PR, HER2 & Ki67). Contrary to the Perou gene set (evolved into PAM50), BluePrint was trained using pathological data.

Substratification of the Luminal subgroup: Concordance MammaPrint versus Ki67
Ki67 is assumed to be a fairly reliable measure of proliferation. Generally, when multi-gene assay results are not available, Ki67 is often used as biomarker to distinguish Luminal A from Luminal B subgroups. The concordance between MammaPrint and centrally assessed Ki67 in Luminal-type patients is 71%, with a +0.26-0.45. The relatively high discordance with MammaPrint indicates that Ki67 and MammaPrint cannot reliably substitute for each other.

Molecular subtyping of HER2+ patients

Methods
Using available data (centrally assessed pathology & genomics) from the MINDACT pilot phase (Rutgers et al, 2011) 621 tumors were analyzed. Patients were classified according to 4-category based pathology (ER, PR, HER2 and Ki67); additionally, classification was done adhering to the recent St. Gallen recommendations (Goldhirsch et al 2011) which recognizes an additional category (Luminal B HER2+). Based on BluePrint 3 subtypes are formed: Luminal, HER2 and Basal. The Luminal subtype is further split into Luminal A (MammaPrint Low Risk) and Luminal B (MammaPrint High Risk).

Conclusions
- All pathological Basal cases are BluePrint Basal, apart from 1 BP HER2 case
  - Of the BluePrint Basal cases, 20% are not pathological Basal (16% Luminal, 4% HER2).
  - Of these 16% Luminal cases, the majority are IHC ER/PR borderline (21% and <10%)
  - 97% of the pathological HER2+ cases that are BluePrint Luminal are ER+
  - Most discordant cases are seen within the Luminal subtype, indicating that Ki67 distinguishes Luminal A from B differently than MammaPrint does

- The observed subtype discrepancies reveal potential important impact for treatment decision making. MINDACT will provide such important information

References
Rutgers et al. 2011, European Journal of Cancer

Acknowledgements
This work was funded through grants from the European Community’Research Infrastructure Actions: L2-P04RRI, the Dutch Cancer Society, the Netherlands Organisation for Scientific Research (NWO), the European Commission under Grant Agreement No. 2612935 (C4C), the European Commission under Grant Agreement No. 226630 (MC-LAG), the National Institutes of Health (Grant 1U10CA102230), the Breast Cancer Research Foundation, the Susan G. Komen Foundation, the American Cancer Society, the Robert J. and Edith M. Dole Human Cancer Research Fund, the National Science Foundation, and the Human Frontier Science Program. The authors declare they have no competing interests.

12 Clinical Luminal patients with BluePrint Basal-type
This figure depicts ER and PR IHC expression for clinical luminal-type samples. BluePrint identifies three subtypes: IHC-Positive, Low Expression IHC (Low); and IHC-negative, and IHC-Positive (High).

The majority of the cases classified as Basal-type by BluePrint have low ER and PR expression (lower than 10%), indicating this to be a critical group in need of further research.

MINDACT and 1 to 5 positive lymph node breast cancer: when may Adjuvant Chemotherapy benefit from ASCO-2012
HER2+ and ER+ are often BP Luminal

- If you have patients with co-morbidities that you are concerned about treating with Herceptin, is there a subset of patients that you can withhold this drug?
- Large group of clinical HER2+ cases that are BluePrint Luminal type (46%).
- Indicating the tumor’s expression of the Luminal profile to be dominate over the expression of the HER2+ profile.
- These patients may have a lower response to trastuzumab (von Minckwitz et al, 2012)

<table>
<thead>
<tr>
<th>4 category</th>
<th>St Gallen (5 category)</th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>HER2</th>
<th>Basal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>ER+ and/or PR+</td>
<td>Luminal A</td>
<td>263</td>
<td>19</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>HER2-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>287</td>
</tr>
<tr>
<td>Luminal B</td>
<td>ER+ and/or PR+</td>
<td>Luminal B HER2-</td>
<td>111</td>
<td>70</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>HER2-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>196</td>
</tr>
<tr>
<td>HER2</td>
<td>ER+ and/or PR+</td>
<td>Luminal B HER2+</td>
<td>25</td>
<td>3</td>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>HER2-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>Basal</td>
<td>ER+/PR+/HER2-</td>
<td>Basal</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>61</td>
</tr>
<tr>
<td>Basal</td>
<td>ER+/PR+/HER2+</td>
<td>Basal</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>61</td>
</tr>
<tr>
<td>Basal</td>
<td>ER-/PR-/HER2-</td>
<td>Basal</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>61</td>
</tr>
<tr>
<td>Basal</td>
<td>ER-/PR-/HER2+</td>
<td>Basal</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>61</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>400</td>
<td>92</td>
<td>53</td>
<td>76</td>
</tr>
</tbody>
</table>
Even the best Ki67 assessment shows 30% discordance with MammaPrint

- Ki67 is assumed to be a fairly reliable measure of proliferation. Ki67 is utilized as a biomarker for chemotherapy.
- The concordance between MammaPrint and centrally assessed Ki67 in Luminal-type patients is 71%, with a k score of 0.35 (95% CI 0.26-0.45).
- The relatively high discordance with MammaPrint indicates that Ki67 and MammaPrint cannot reliably substitute for each other.
- MammaPrint has a higher hazard ratio than Ki67 and is a better indicator for prognosis.
Key Findings:
20% of the Basal is IHC ER+

- These patients might take Endocrine therapy without effect
- Of the BluePrint Basal cases, 20% are not pathological Basal (16% Luminal, 4% HER2+)
- Of the 16% Luminal cases, the majority (80% are IHC ER/PR borderline (≥ 1% and < 10%)

<table>
<thead>
<tr>
<th>4 category</th>
<th>St Gallen (5 category)</th>
<th>Luminal A (BluePrint Luminal Mammaprint: low risk)</th>
<th>Luminal B (BluePrint Luminal Mammaprint: high risk)</th>
<th>HER2 (BluePrint HER2)</th>
<th>Basal (BluePrint Basal)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>ER+ and/or PR+ HER2-, Ki67 low</td>
<td>263</td>
<td>19</td>
<td>4</td>
<td>1</td>
<td>287</td>
</tr>
<tr>
<td>Luminal B</td>
<td>ER+ and/or PR+ HER2-, Ki67 high</td>
<td>111</td>
<td>70</td>
<td>4</td>
<td>11</td>
<td>196</td>
</tr>
<tr>
<td>HER2</td>
<td>HER2+</td>
<td>25</td>
<td>3</td>
<td>31</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Erb-B2</td>
<td>1</td>
<td>0</td>
<td>13</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>Basal</td>
<td>(ER+/PR+/HER2+)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>61</td>
<td>62</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>400</td>
<td>92</td>
<td>53</td>
<td>76</td>
<td>621</td>
</tr>
</tbody>
</table>
Are clinico-pathological data useful in “fine tuning” your treatment decisions towards adjuvant chemotherapy?

Then the basic & confusing data….
Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials

Early Breast Cancer Trialists' Collaborative Group (EBCTCG)

Summary

Background: Moderate differences in efficacy between adjacent chemotherapy regimens for breast cancer are plausible.

Figure 5: Time to recurrence, breast cancer mortality, and overall mortality for chemotherapy versus no adjuvant chemotherapy. Left: four or more cycles of any anthracycline (Anthr)-based regimen—eg, standard 4AC. Right: standard or near-standard CMF. RR (and its 95% CI)—event rate ratio, from summed log-rank statistics for all time periods combined. Gain (and its SE)—absolute difference between ends of graphs. CTX—chemotherapy. Event rates, %/year, are followed by (first events/woman-years). Error bars show ±1 SE.

Figure 6: Subgroup analyses of breast cancer mortality (event rate with recurrence, by log-rank method) among any anthracycline-based regimen versus no chemotherapy.
Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100 000 women in 123 randomised trials

Early Breast Cancer Trials’ Collaborative Group (EBCTCG)

Summary

Background Moderate differences in efficacy between adjuvant chemotherapy regimens for breast cancer are plausible.

(F) ER status ($\chi^2=0.1; 2p=0.7; \text{NS}$)

<table>
<thead>
<tr>
<th></th>
<th>Count</th>
<th>%</th>
<th>Count</th>
<th>%</th>
<th>Odds Ratio</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-poor</td>
<td>403/1095 (36.8%)</td>
<td>464/1043 (44.5%)</td>
<td>-40.5</td>
<td>180.4</td>
<td>0.80 (SE 0.07)</td>
<td></td>
</tr>
<tr>
<td>ER+</td>
<td>831/3100 (26.8%)</td>
<td>1063/3177 (33.5%)</td>
<td>-84.6</td>
<td>328.5</td>
<td>0.77 (SE 0.05)</td>
<td></td>
</tr>
<tr>
<td>ER unknown</td>
<td>182/559 (32.6%)</td>
<td>174/513 (33.9%)</td>
<td>-14.9</td>
<td>72.3</td>
<td>0.81 (SE 0.11)</td>
<td></td>
</tr>
</tbody>
</table>

Subsets of ER+

<table>
<thead>
<tr>
<th>Subset</th>
<th>Count</th>
<th>%</th>
<th>Count</th>
<th>%</th>
<th>Odds Ratio</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+, chemotherapy+endocrine vs endocrine</td>
<td>659/2622 (25.1%)</td>
<td>853/2675 (31.9%)</td>
<td>-56.2</td>
<td>247.0</td>
<td>0.80 (SE 0.06)</td>
<td></td>
</tr>
<tr>
<td>ER 10-99 fmol/mg</td>
<td>416/1371 (30.3%)</td>
<td>544/1442 (37.7%)</td>
<td>-35.3</td>
<td>162.5</td>
<td>0.80 (SE 0.07)</td>
<td></td>
</tr>
<tr>
<td>ER ≥100 fmol/mg</td>
<td>274/1146 (23.9%)</td>
<td>337/1160 (29.1%)</td>
<td>-20.6</td>
<td>95.6</td>
<td>0.81 (SE 0.09)</td>
<td></td>
</tr>
<tr>
<td>ER+, age &lt;55 years</td>
<td>250/845 (29.6%)</td>
<td>316/943 (33.5%)</td>
<td>-19.4</td>
<td>102.4</td>
<td>0.83 (SE 0.09)</td>
<td></td>
</tr>
<tr>
<td>ER+, age 55–69 years</td>
<td>542/2071 (26.2%)</td>
<td>677/2055 (32.9%)</td>
<td>-53.9</td>
<td>215.3</td>
<td>0.78 (SE 0.06)</td>
<td></td>
</tr>
<tr>
<td>ER+, poorly differentiated</td>
<td>100/461 (21.7%)</td>
<td>120/477 (25.2%)</td>
<td>-12.2</td>
<td>45.8</td>
<td>0.77 (SE 0.13)</td>
<td></td>
</tr>
<tr>
<td>ER+, moderately/well differentiated</td>
<td>228/985 (23.1%)</td>
<td>286/1026 (27.9%)</td>
<td>-27.8</td>
<td>112.8</td>
<td>0.78 (SE 0.08)</td>
<td></td>
</tr>
</tbody>
</table>
## Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials

*Early Breast Cancer Trials’ Collaborative Group (EBCTCG)*

### Summary

Background: Moderate differences in efficacy between adjuvant chemotherapy regimens for breast cancer are plausible.

### Table: Deaths/women

<table>
<thead>
<tr>
<th></th>
<th>Allocated Anthracycline</th>
<th>Allocated CMF</th>
<th>Anthracycline deaths</th>
<th>Ratio of annual death rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Log-rank O-E</td>
<td>Variance of O-E</td>
</tr>
<tr>
<td>(A) Cumulative anthracycline dosage, if dose per cycle is at least 60mg/m² (trend x²: 0.9; 2p = 0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age or Coxsibs mg/m² (AVF)</td>
<td>217/220/23 (18.7%)</td>
<td>417/220 (25.1%)</td>
<td>180.0</td>
<td>180.0</td>
</tr>
<tr>
<td>Age or Euro-ain</td>
<td>250/220 (19.7%)</td>
<td>470/220 (22.4%)</td>
<td>119.9</td>
<td>119.9</td>
</tr>
<tr>
<td>Age or standard MAC</td>
<td>257/220 (18.3%)</td>
<td>470/220 (22.4%)</td>
<td>212.1</td>
<td>212.1</td>
</tr>
<tr>
<td>Doxorubicin:Alcoho</td>
<td>185/220 (24.8%)</td>
<td>375/220 (22.3%)</td>
<td>-11.1</td>
<td>100.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B) Cyclophosphamide in CMF trials (x²=0.9; 2p=0.3; NS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concomitant alkyl</td>
<td>156/220 (25.3%)</td>
<td>475/220 (22.4%)</td>
<td>-98.0</td>
<td>718.9</td>
</tr>
<tr>
<td>Concomitant taxol</td>
<td>156/220 (25.3%)</td>
<td>455/220 (22.4%)</td>
<td>-98.0</td>
<td>718.9</td>
</tr>
<tr>
<td>(C) Concurrent endocrine therapy (6ER+ x²=0.9; 2p=0.3; NS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>57/220 (25.1%)</td>
<td>317/220 (22.4%)</td>
<td>2.4</td>
<td>6.4</td>
</tr>
<tr>
<td>No (any endocrine only after chemotherapy ended)</td>
<td>156/220 (25.3%)</td>
<td>475/220 (22.4%)</td>
<td>2.4</td>
<td>6.4</td>
</tr>
<tr>
<td>(D) Entry age (trend x²: 0.9; 2p=0.3; NS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45 years</td>
<td>87/130/26 (25.6%)</td>
<td>501/130/26 (22.4%)</td>
<td>-58.4</td>
<td>482.8</td>
</tr>
<tr>
<td>45-54 years</td>
<td>76/130/26 (23.7%)</td>
<td>301/130/26 (22.4%)</td>
<td>-30.6</td>
<td>344.3</td>
</tr>
<tr>
<td>55-64 years</td>
<td>25/130/26 (20.1%)</td>
<td>256/130/26 (22.4%)</td>
<td>-29.2</td>
<td>159.3</td>
</tr>
<tr>
<td>&gt;65 years</td>
<td>18/130/26 (18.5%)</td>
<td>25/130/26 (22.4%)</td>
<td>-2.2</td>
<td>8.7</td>
</tr>
<tr>
<td>Unknown</td>
<td>7/130/26 (6.9%)</td>
<td>7/130/26 (6.9%)</td>
<td>2.4</td>
<td>6.4</td>
</tr>
<tr>
<td>(E) Nodal status (trend x²: 0.9; 2p=0.3; NS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0+</td>
<td>46/130/26 (15.6%)</td>
<td>541/130/26 (24.4%)</td>
<td>-24.9</td>
<td>333.1</td>
</tr>
<tr>
<td>N1-</td>
<td>520/130/26 (21.7%)</td>
<td>543/130/26 (24.4%)</td>
<td>-10.0</td>
<td>243.4</td>
</tr>
<tr>
<td>N2+</td>
<td>612/130/26 (46.9%)</td>
<td>647/130/26 (27.5%)</td>
<td>-33.3</td>
<td>273.4</td>
</tr>
<tr>
<td>Other/unknown</td>
<td>46/130/26 (15.6%)</td>
<td>459/130/26 (24.4%)</td>
<td>-21.9</td>
<td>238.8</td>
</tr>
<tr>
<td>(F) HR status (trend x²: 0.9; 2p=0.3; NS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-</td>
<td>126/490/1400 (26.8%)</td>
<td>1200/490/1400 (28.5%)</td>
<td>-13.7</td>
<td>504.5</td>
</tr>
<tr>
<td>ER+</td>
<td>540/490/1400 (28.7%)</td>
<td>2600/490/1400 (28.5%)</td>
<td>26.5</td>
<td>564.5</td>
</tr>
<tr>
<td>ER unknown</td>
<td>236/490/1400 (20.3%)</td>
<td>236/490/1400 (20.3%)</td>
<td>-35.2</td>
<td>115.2</td>
</tr>
<tr>
<td>Subsets of ER+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-50-90% lossing</td>
<td>240/1400/1400 (16.3%)</td>
<td>1400/1400/1400 (12.0%)</td>
<td>-15.2</td>
<td>108.0</td>
</tr>
<tr>
<td>ER-100% lossing</td>
<td>146/1400/1400 (14.7%)</td>
<td>1400/1400/1400 (10.5%)</td>
<td>-15.4</td>
<td>41.9</td>
</tr>
<tr>
<td>ER+, age &lt;55 years</td>
<td>136/1400/1400 (22.1%)</td>
<td>1400/1400/1400 (16.0%)</td>
<td>-17.7</td>
<td>203.2</td>
</tr>
<tr>
<td>ER+, age &gt;55 years</td>
<td>136/1400/1400 (22.1%)</td>
<td>1400/1400/1400 (16.0%)</td>
<td>-17.7</td>
<td>203.2</td>
</tr>
<tr>
<td>ER+, poor differentiation</td>
<td>136/1400/1400 (22.1%)</td>
<td>1400/1400/1400 (16.0%)</td>
<td>-17.7</td>
<td>203.2</td>
</tr>
<tr>
<td>ER+, moderately/well differentiated</td>
<td>136/1400/1400 (22.1%)</td>
<td>1400/1400/1400 (16.0%)</td>
<td>-17.7</td>
<td>203.2</td>
</tr>
<tr>
<td>Total</td>
<td>2000/490/1400 (23.5%)</td>
<td>2100/490/1400 (23.5%)</td>
<td>-16.4</td>
<td>946.8</td>
</tr>
</tbody>
</table>

- 95% CI or 95% CI
- Global heterogeneity χ²: 0.9; p=0.1
- χ²: 0.0066
- CMF CI
- Anthracycline CI

---

Figure 4: Subgroup analyses of breast cancer mortality (mortality with recurrence, by log-rank subtraction) for any anthracyline-based regimen versus standard CMF (or near-standard CMF)

A = doxorubicin; E = epirubicin; Doxorubicin (cumulative dose) is given after the drug name in mg/m²; Alcoho = means 60 mg/m² of doxorubicin or 50 mg/m² of epirubicin. NS = not significant. ER = oestrogen receptor. HR = immunohistochemistry. First four subgroups are as in the forest plots (with appendix pp. 2–7) that give details of each trial’s oestrogen regimens.

Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials

Early Breast Cancer Trials’ Collaborative Group (EBCTCG)

Summary

Background: Moderate differences in efficacy between adjuvant chemotherapy regimens for breast cancer are plausible.

(F) ER status ($\chi^2=0.1; 2p=0.8; NS$)

<table>
<thead>
<tr>
<th>ER status</th>
<th>Count/Total</th>
<th>Count/Total</th>
<th>Count/Total</th>
<th>Count/Total</th>
<th>Count/Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-poor</td>
<td>120/4488 (26.3%)</td>
<td>1287/4518 (28.5%)</td>
<td>-437</td>
<td>564.6</td>
<td>0.93 (SE 0.04)</td>
</tr>
<tr>
<td>ER+</td>
<td>569/3279 (17.4%)</td>
<td>610/3257 (18.7%)</td>
<td>-265</td>
<td>267.0</td>
<td>0.91 (SE 0.06)</td>
</tr>
<tr>
<td>ER unknown</td>
<td>239/1116 (20.3%)</td>
<td>293/1151 (25.5%)</td>
<td>-35.2</td>
<td>115.2</td>
<td>0.74 (SE 0.08)</td>
</tr>
</tbody>
</table>

Subsets of ER+

<table>
<thead>
<tr>
<th>Subset</th>
<th>Count/Total</th>
<th>Count/Total</th>
<th>Count/Total</th>
<th>Count/Total</th>
<th>Count/Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER 10-99 fmol/mg</td>
<td>247/1072 (23.0%)</td>
<td>279/1094 (25.5%)</td>
<td>-21.2</td>
<td>108.3</td>
<td>0.82 (SE 0.09)</td>
</tr>
<tr>
<td>ER ≥100 fmol/mg</td>
<td>85/450 (19.1%)</td>
<td>116/450 (25.8%)</td>
<td>-15.4</td>
<td>42.0</td>
<td>0.69 (SE 0.13)</td>
</tr>
<tr>
<td>ER+, age &lt;55 years</td>
<td>425/2359 (18.1%)</td>
<td>461/2345 (19.7%)</td>
<td>-22.9</td>
<td>202.3</td>
<td>0.89 (SE 0.07)</td>
</tr>
<tr>
<td>ER+, 55-69 years</td>
<td>134/845 (15.8%)</td>
<td>140/847 (16.5%)</td>
<td>-3.6</td>
<td>61.1</td>
<td>0.94 (SE 0.12)</td>
</tr>
<tr>
<td>ER+, poorly differentiated</td>
<td>131/968 (15.1%)</td>
<td>130/793 (16.4%)</td>
<td>-41</td>
<td>52.7</td>
<td></td>
</tr>
<tr>
<td>ER+, moderately/well</td>
<td>125/952 (13.1%)</td>
<td>136/1047 (13.0%)</td>
<td>-18</td>
<td>58.3</td>
<td></td>
</tr>
</tbody>
</table>
Value of molecular subtyping and prediction to effect of chemotherapy

- Classification often dependent on method used
- Despite differences in gene lists, outcome similar
- Most signatures discriminate based on ER-status and proliferative activity
- Prognostic value restricted to ER-positive tumors
- Subclassification ER-positive breast cancer in luminal A and luminal B is arbitrary, based on proliferation
- Expression signatures are complementary to standard clinico-pathological parameters

Weigelt et al., Nature Reviews Clinical Oncology (2011)
Predictive profiles fail

- Even the best arrays unable to give a sufficient signal at low expression of very relevant genes
- Subtle, non-detectable changes in level of expression can make the difference
- Expression profiling unable to pick up resistance mechanisms if such a mechanism is only present in a proportion of the tumors
- Tumors are heterogeneous, RNA bulk analysis will not help

Borst & Wessels, Cell Cycle (2011) 9: 4836
Integration of tumor features is essential

- Adequate morphological diagnosis
- Robust and reliable IHC-panel
  - ER, PR, HER2, (Ki-67)
- Gene signature has additional value for a substantial subgroup
- Requires for all disciplines sufficient volume and expertise
The clinical issue.
Think step by step
(the most difficult one)

Step 4: What are the factors that justify adjuvant chemotherapy (luminal A > B)

My conclusions (for debate)

- Is every luminal A a luminal A? No, there are some high risk cancers between them
- What makes luminal B a luminal B? The proliferation/propensity to disseminate: you need extra information because you do not see it sufficiently on standard pathology/IHC (Ki 67: too much differences in quality, too many ‘in betweens’, not proven to be chemopredictive).
The clinical issue.
Think step by step
(the most difficult one)

Step 4: What are the factors that justify adjuvant chemotherapy (luminal A > B)
My conclusions (for debate)

• What is the effect of chemotherapy: different for luminal A or B?
  Actually not proven: depends on prognosis & prediction
  (and now, I’m sorry, the circle is round again)
Did you get some order out of chaos?
Thanks to the patients and all those who provided me with the presented information, and for inviting me, your attention & discussion.
Intermediate Clinico/Pathological Risk

What to do?

• Treat all patients with chemotherapy?
• Or be more selective, and treat those patients who benefit

(and thus minimizing the risk of losing lives by foregoing chemotherapy)
70-gene assay (MammaPrint)

• Is not just another prognostic factor
• Is designed from the beginning to tell you the metastatic potential of an individual breast cancer
70-gene MammaPrint

- Functions of many genes are identified and are all related to the process of dissemination including proliferation
Validation 4: N = 100

Wittner et al., Clin Cancer Res 14: 2988, 2008

MGH series, Boston;
Time to metastasis

Probability of remaining metastasis-free

Years

p = 0.123
Aims RASTER study

• Feasibility of using 70-gene signature in community-based settings

• Effect of 70-gene signature on adjuvant systemic treatment (AST) decisions
  – AST decision at that time based on restrictive Dutch National Guideline 2004, 70GS result and doctors’ and patients’ preferences

• Outcome after 5 and 10 years of follow-up
Current aim RASTER study

- Outcome after 5 years of follow-up
- What would the risk estimation of the RASTER cohort be with currently used risk estimation tools to guide AST decisions

– Adjuvant!Online
Inclusion criteria

- Female
- cT1-4N0M0 invasive breast cancer
- Age < 61 years, amended to < 55 years (after 242 patients had been enrolled)
- Operable, unilateral tumor
- No history of previous malignancy, except for basal cell carcinoma or cervical carcinoma in situ
- No neoadjuvant systemic therapy
High risk

- N+

- N0; ≤35 years
  - except for tumor ≤ 1 cm grade I

- N0; > 35 years:
  - Larger than 1 cm grade III
  - Larger than 2 cm grade II
  - Larger than 3 cm any grade
Low risk defined as 10-year survival probability at least 90%

50 years

9% 10-yrs † risk

Benefit ET 3%, CT 2%

www.adjuvantonline.com
Results

- 427 patients tested between 2004-2006
- Median FUP time 61.6 months
- 33 DDFS events
  - DDFS event = distant recurrence, death (any cause), 2nd primary other than breast
- 11 deaths
- 9 breast cancer specific deaths
Proportion of patients labeled as high risk

- 70-gene signature:
  - High risk: 208 (49%)
  - Low risk: 219 (51%)

- Adjuvant Online:
  - High risk: 295 (69%)
  - Low risk: 132 (31%)
With 70GS 29% less patients high risk category, compared to AOL.

- 70-gene signature:
  - High risk: 208 (49%)
  - Low risk: 219 (51%)

- Adjuvant Online:
  - High risk: 295 (69%)
  - Low risk: 132 (31%)
5-year DDFS of 427 patients according to 70GS or AOL

- **70-gene signature**
  - High risk: 208 (49%)
  - Low risk: 219 (51%)
  - 5YR DDFS: 89.8%

- **Adjuvant Online**
  - High risk: 295 (69%)
  - Low risk: 132 (31%)
  - 5YR DDFS: 92.4%
70GS-AOL risk groups and AST

- Concordant low risk
- 70GS high-AOL low
- 70GS low-AOL high
- Concordant high risk

Legend:
- CT & ET
- ET
- CT
- no AST
## Patient characteristics discordant group

**n=94 patients no AST or ET only**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 45-55 years</td>
<td>75%</td>
</tr>
<tr>
<td>pT1 (&lt; 2 cm)</td>
<td>80%</td>
</tr>
<tr>
<td>Grade II</td>
<td>82%</td>
</tr>
<tr>
<td>IDC / ILC</td>
<td>72 / 20%</td>
</tr>
<tr>
<td>ER pos</td>
<td>98%</td>
</tr>
<tr>
<td>PgR pos</td>
<td>78%</td>
</tr>
<tr>
<td>HER neg</td>
<td>90%</td>
</tr>
</tbody>
</table>
Conclusions

• AOL high risk and 70GS low risk patients who did not receive adjuvant systemic therapy or hormonal therapy only had an excellent 5-year DDFS (97.8%)

• This percentage is unlikely to drop below 90% at 10 years of follow-up

• Of this patient group at least 80% had an ER positive, HER2 negative, grade II tumor of 1 to 2 cm in size

• The percentage of high risk patients could be reduced by almost 30% when 70GS risk estimation was used
Acknowledgements

All participating patients
Multiple answers from a single array

MammaPrint
70 x 9
231 x 5

Molecular subtypes
BluePrint
80 x 5

mRNA readout ER, PR and HER2
TargetPrint
3 x 5

Research Gene Panel
TheraPrint
56 x 3

Normalization
465 x 3
Control probes
536

Drug response profile
Drug response profile