Molecular Classification of Breast Cancer – Implications for Therapy Selection

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Professor Medicine, UBC
Medical Oncologist, BC Cancer Agency
Summary

• Describe the current molecular classifications of breast cancer
• Discuss the impact of the molecular classifications on treatment decisions
• Describe some of the new classifications that are being described
• Discuss the implications for future trials
• Improvements partly due to screening, partly due to better Rx
• Improvements in outcome ER+ > HER2+ > TNBC
• How do we continue this trend?

Adapted from Peto R et al, Lancet 2000
DNA MICROARRAY GENE EXPRESSION PROFILING REVEALS SIGNATURES OF BREAST CANCER SUBTYPES WITH PROGNOSTIC VALUE

The Major Breast Cancer Intrinsic Biological Subtypes

<table>
<thead>
<tr>
<th></th>
<th>Lum A</th>
<th>Lum B</th>
<th>HER2</th>
<th>basal</th>
</tr>
</thead>
</table>
| **Estrogen Response genes:**  
  *ESR1, PGR, GATA3, FOXA1*  | +     | +     | -    | -     |
| **Proliferation genes:**  
  *MKI67, CCNB1, CENPF, FOXA1, MYBL2, ORC6L*  | -     | +     | +    | +     |
| **HER2-associated:**  
  *ERBB2, GRB7*  | -     | +/-   | +    | -     |
| **Basal markers:**  
  *KRT5, KRT17, ERBB1, TRIM29, CRYAB*  | -     | -     | -    | +     |
# IMMUNOSTAIN PANEL FOR BREAST CANCER SUBTYPING

<table>
<thead>
<tr>
<th>ER</th>
<th>PR</th>
<th>HER2</th>
<th>EGFR</th>
<th>CK5/6</th>
<th>Ki67</th>
<th>Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td>Luminal A TAM/AI (or none)</td>
</tr>
<tr>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
<td>Luminal B TAM/AI + chemo</td>
</tr>
<tr>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
<td><img src="image15.png" alt="Image" /></td>
<td><img src="image16.png" alt="Image" /></td>
<td><img src="image17.png" alt="Image" /></td>
<td><img src="image18.png" alt="Image" /></td>
<td>HER2 trastuzumab + chemo</td>
</tr>
<tr>
<td><img src="image19.png" alt="Image" /></td>
<td><img src="image20.png" alt="Image" /></td>
<td><img src="image21.png" alt="Image" /></td>
<td><img src="image22.png" alt="Image" /></td>
<td><img src="image23.png" alt="Image" /></td>
<td><img src="image24.png" alt="Image" /></td>
<td>Basal chemo ... ?best type</td>
</tr>
</tbody>
</table>

Immunohistochemical surrogate panel derived from gene expression profile data allows molecular subtyping of breast cancers on tissue microarrays.


**Gene Expression Profiling of Breast Cancer**

Maggie C.U. Cheung,¹ Mart van de Rijn,² and Torsten O. Nielsen¹
## Identifying the molecular subtype

<table>
<thead>
<tr>
<th>standard clinical test</th>
<th>ER+ HER2-</th>
<th>ER+ HER2+</th>
<th>ER- HER2+</th>
<th>ER- HER2-</th>
</tr>
</thead>
<tbody>
<tr>
<td>additional IHC</td>
<td>Ki67 low</td>
<td>Ki67 high</td>
<td>HER2</td>
<td>EGFR or ck5+</td>
</tr>
<tr>
<td>Mamma-Print (array)</td>
<td>good prog</td>
<td></td>
<td>poor prognosis</td>
<td></td>
</tr>
<tr>
<td>Oncotype (qRT-PCR)</td>
<td>low RS</td>
<td></td>
<td>high Recurrence Score</td>
<td></td>
</tr>
<tr>
<td>Intrinsic / PAM50</td>
<td>Lum A</td>
<td>Lum B</td>
<td>HER2</td>
<td>basal</td>
</tr>
</tbody>
</table>
### Identifying the molecular subtype

<table>
<thead>
<tr>
<th>method</th>
<th>Cost in $$</th>
<th>FFPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>standard testing</td>
<td>0</td>
<td>yes</td>
</tr>
<tr>
<td>additional IHC</td>
<td>40</td>
<td>yes</td>
</tr>
<tr>
<td>MammaPrint (microarray)</td>
<td>4000</td>
<td>no (requires frozen tissue)</td>
</tr>
<tr>
<td>OncotypeDX (qRT-PCR)</td>
<td>4000</td>
<td>yes</td>
</tr>
<tr>
<td>PAM50 (Nanostring)</td>
<td>400</td>
<td>yes</td>
</tr>
<tr>
<td>next generation sequencing</td>
<td>5-10,000</td>
<td>yes recently</td>
</tr>
</tbody>
</table>
Predicting Outcomes by Subtype

- Prognostic
- Subtype variation with risk of local relapse
- Variation by subtype of sites of mets
- Prediction of response to neoadjuvant therapy
- Prediction of response to specific treatments including specific chemotherapy

- BUT most of the data is retrospective
Biological subtypes of breast cancer

**CLINICAL SUBTYPES**

- HER2- ER-
  - Express Basal markers
  - Younger patients
  - Fewer therapeutic options
- HER2- ER-
  - Non basal
- ER- ~35%
  - ~15%
  - HER2+ trastuzumab
- ER+ ~65%
  - Special types
  - High mitotic rate ER+
  - Low mitotic rate ER+
  - Endocrine therapy +/- cytotoxic chemo

**MOLECULAR SUBTYPES**

Eg: **PAM50**
50-gene transcript expression based centroids for classification

- HER2=HER2 (ER+ or ER-)
- LUMA=ER+, low mitotic rate
- LUMB=ER+, mitotic active
- BASAL=subtype of “triple negative”
- Normal-like="similar to normal breast epithelium"

![Graph showing Kaplan-Meier curves for Breast Cancer](chart.png)

(Parker et al, JCO 2009)
Breast Cancer Subtypes and the Risk of Local and Regional Relapse

K. David Voduc, Maggie C.U. Cheang, Scott Tyldesley, Karen Gelmom, Torsten O. Nielsen, and Hagen Kennecke

See accompanying editorial doi: 10.1200/JCO.2009.27.1080

intrinsic subtype is also prognostic for local and regional relapses, post lumpectomy and post mastectomy...
The metastatic behavior of breast cancer subtypes

*Kennecke H et al., J Clin Oncol, 2010*

<table>
<thead>
<tr>
<th>subtype</th>
<th>median survival w/ mets (yr)</th>
<th>most common site of met</th>
<th>other sites @↑risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lum A</td>
<td>2.2</td>
<td>bone</td>
<td></td>
</tr>
<tr>
<td>Lum B</td>
<td>1.6</td>
<td>bone</td>
<td></td>
</tr>
<tr>
<td>Lum/HER2</td>
<td>1.3</td>
<td>bone</td>
<td>brain, liver, lung</td>
</tr>
<tr>
<td>HER2E</td>
<td>0.7</td>
<td>bone</td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td>0.5</td>
<td>lung</td>
<td>brain</td>
</tr>
</tbody>
</table>
Biology of Breast Cancer:
Multiple Distinct Subtypes

Luminal A
Luminal B
Claudin-low
Basal-like
HER2-enriched

Primary therapy = endocrine

Chemotherapy is mainstay

Triple negative” (ER, PR, HER2) on clinical assays
HER2+ on clinical assays (can be ER + or -)

Courtesy Chuck Perou
Pathologic Response to Chemotherapy by Subtype

Modified PAM50 subtyping in 360 patients treated with anthracycline/taxane chemotherapy only (no trastuzumab!)
Overall pCR rate = 22%

<table>
<thead>
<tr>
<th>Classification</th>
<th>Residual disease</th>
<th>Pathologic complete response (pCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal-like</td>
<td>47 (58%)</td>
<td>34 (42%)</td>
</tr>
<tr>
<td>Claudin-low</td>
<td>29 (67%)</td>
<td>14 (33%)</td>
</tr>
<tr>
<td>HER2-enriched</td>
<td>31 (63%)</td>
<td>18 (37%)</td>
</tr>
<tr>
<td>LumA</td>
<td>110 (98%)</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>LumB</td>
<td>56 (85%)</td>
<td>10 (15%)</td>
</tr>
<tr>
<td>Normal-like</td>
<td>13 (76%)</td>
<td>4 (24%)</td>
</tr>
</tbody>
</table>

Courtesy C. Perou
CALGB 9344 / INT 0148

Node Positive Breast Cancer (n=3121)
all: radiation as indicated; tamoxifen 20mg/day X 5 yrs if ER positive

Disease Free Survival

P<0.001

adjuvant AC (doxorubicin + cyclophosphamide),
randomized to added paclitaxel q3wk or not

2003; 976-83 = 5% better DFS in paclitaxel arm
Paclitaxel in HER2+, ER- breast cancer (N = 221)

In C9344, added paclitaxel benefits women with HER2+/ER- breast cancer

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-value</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Positive Nodes (square root)</td>
<td>&lt;.0001</td>
<td>1.65</td>
<td>1.36-2.01</td>
</tr>
<tr>
<td>Tumor Size (&lt;= 2 vs &gt;2 cm)</td>
<td>0.70</td>
<td>1.09</td>
<td>0.71-1.68</td>
</tr>
<tr>
<td>Age</td>
<td>0.66</td>
<td>1.00</td>
<td>0.98-1.01</td>
</tr>
<tr>
<td>Paclitaxel Treatment</td>
<td>0.0032</td>
<td>0.57</td>
<td>0.39-0.83</td>
</tr>
</tbody>
</table>

Proportion Relapse-free

HER+/ER- subset by Paclitaxel
Paclitaxel in **core basal** breast cancer (N = 444)

**Relapse-free Survival**

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-value</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Positive Nodes (square root)</td>
<td>&lt;.0001</td>
<td>1.47</td>
<td>1.28-1.67</td>
</tr>
<tr>
<td>Tumor Size (&lt;= 2 vs &gt;2 cm)</td>
<td>0.018</td>
<td>1.45</td>
<td>1.06-1.96</td>
</tr>
<tr>
<td>Age</td>
<td>0.022</td>
<td>1.02</td>
<td>1.00-1.03</td>
</tr>
<tr>
<td>Paclitaxel Treatment</td>
<td>0.0033</td>
<td>0.75</td>
<td>0.58-0.98</td>
</tr>
</tbody>
</table>

In C9344, added paclitaxel benefits women with basal-like breast cancer

*n.b.: double negative definition (N=557) has paclitaxel HR 0.8, p=0.07*
Paclitaxel in **Luminal A** breast cancer (N = 790)

In C9344, there is no benefit of added paclitaxel among women with Luminal A breast cancer.
Paclitaxel in **Luminal B**

breast cancer (N = 340)

### Variable Analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-value</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Positive Nodes</td>
<td>&lt;.0001</td>
<td>1.57</td>
<td>1.34-1.83</td>
</tr>
<tr>
<td>Tumor Size (&lt;= 2 vs &gt;2 cm)</td>
<td>0.13</td>
<td>1.30</td>
<td>0.93-1.81</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;.0001</td>
<td>0.97</td>
<td>0.95-0.98</td>
</tr>
<tr>
<td>Paclitaxel Treatment</td>
<td>0.018</td>
<td>0.69</td>
<td>0.51-0.94</td>
</tr>
</tbody>
</table>

*In C9344, added paclitaxel benefits women with Luminal B breast cancer*
Breast Cancer Subtypes and Response to Docetaxel in Node-Positive Breast Cancer: Use of an Immunohistochemical Definition in the BCIRG 001 Trial

Judith Hugh, John Hansen, Maggie Chon U. Cheang, Torsten O. Nielsen, Charles M. Perou, Charles Dumontet, John Reed, Maryla Krajewska, Isabelle Treilleux, Matthieu Rupin, Emmanuelle Magherini, John Mackey, Miguel Martin, and Charles Vogel

Published Ahead of Print on February 9, 2009 as 10.1200/JCO.2008.18.1024

N=1491
Luminal Subtypes

- Hormonal Agents remain the mainstay but there has been difficulty in terms of other targets
- Oncotype DX to sort out addition of chemotherapy
- PAM50 and subtype to sort out chemotherapy
- All the data is currently retrospective
- Which chemotherapy?
- Addition of taxanes for luminal B
- Questions of other targets
- MTOR, IGFR, HER family
What about hormone therapy alone?
Cohort: N = 770 ER+, tamoxifen-treated breast cancers from BC: outcomes by PAM50 assay
Comparison of PAM50 risk of recurrence (ROR) score with OncotypeDx and IHC4 for predicting residual risk of recurrence and distant recurrence after endocrine therapy: a TransATAC study.

Dowsett¹, Ivana Sestak⁴, Elena Lopez-Knowles¹, Kally Sidhu¹, J. Wayne Cowens², Sean Ferree², James Storhoff², Carl Schaper³, Jack Cuzick⁴
on behalf of the ATAC Trialists’ Group

1. Royal Marsden Hospital, London, UK
2. NanoString Technologies, Seattle, USA
3. Myraqa, Redwood City, USA
4. Wolfson Inst for Preventive Medicine, London, UK
Capture patient expression profile
Extract RNA from FFPE tumor sample
Run RNA & PAM50 CodeSet on nCounter Analysis System

Determine Intrinsic Subtype through Pearson’s Correlation to Centroids

ROR = aR_{LumA+}
  + bR_{LumB+}
  + cR_{Her2e+}
  + dR_{Basal+}
  + eP+
  + fT

Calculate Risk of Recurrence (ROR) Score

Proliferation score (19 genes)
Does the ROR score add prognostic information for Distant Recurrence over and above the Clinical Treatment Score?

<table>
<thead>
<tr>
<th>Analyses</th>
<th>Population</th>
<th>No. Patients</th>
<th>ΔLR-χ²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>All Evaluable</td>
<td>1007</td>
<td>33.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Node Negative</td>
<td>739</td>
<td>24.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Secondary</td>
<td>Node Positive</td>
<td>268</td>
<td>9.3</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>HER2 Negative</td>
<td>888</td>
<td>28.0</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

ROR is significantly related to outcome for all populations tested.
Kaplan Meier curves of Luminal A vs. Luminal B by nodal status

Node Negative Patients
- Luminal A (529)
- Luminal B (176)

Node Positive Patients
- LumA, N1-3 (132) LumB, N1-3 (69)
- LumA, N ≥4 (31) LumB, N ≥4 (20)

HR=4.78 (2.97-7.70)
HR (N1-3)=2.20 (1.10-3.61) HR (N≥4) =3.40 (1.60-7.22)
Summary of the PAM50 and ROR analysis of TransATAC

• The PAM50 ROR score was significantly related to the probability of 10 year distant recurrence

• The ROR score added prognostic information beyond the Clinical Treatment Score in:
  • all patients
  • node-negative patients
  • node-positive patients
  • HER2-negative patients

• The breast cancer intrinsic subtypes, Luminal A and Luminal B, have significantly different outcomes when treated with endocrine therapy alone
Triple Negative Breast Cancer

Made up of:
- Basal-like (red)
  - Majority
  - Low HR, HER2 genes
  - High proliferation gene
- Claudin-low (yellow)
  - Minority
  - Low HR, HER2 genes
  - Relatively low proliferation genes
- ~20% Luminal or HER2-enriched subtypes
- Mainstay of therapy = chemotherapy
TNBC comprised of diverse molecular subtypes

Validation ongoing
Affymetrix gene expression profiling of FFPE samples
Intrinsic subtypes assigned using Sorlie et al, PNAS, 2003 data set and claudin-low classifier (Prat et al., BCR, 2010) [courtesy of J. Theilhaber and D. Bergstrom, Sanofi]
Heterogeneity of Triple Negative Targets

- Genomic instability hallmark
- Multiple subclusters with varying targets
  - Basal-like 1 and 2 – DNA damage response genes
  - Immunomodulatory
  - Mesenchymal and mesenchymal / stem cell – PI3K/mTOR pathway but no activity seen in Gepartrio substudy
  - LAR – androgen receptor

Lehmann et al, JCI 2011
Prognostic factors in Basal Breast Cancers

Fig. 1: $\alpha$-basic crystallin and breast cancer-specific survival

**All invasive breast cancers**

- Negative: N = 2870
- Positive: N = 359

Hazard ratio: 1.30 (95% CI 1.04-1.62), p = 0.022*
*adjusted for age, LVI, nodal status, grade, tumor size, and breast cancer subtypes

**Among Basal-like tumors**

- Negative: N = 139
- Positive: N = 170

Hazard ratio: 1.50 (95% CI 1.01-2.25), p = 0.047*
*adjusted for age, LVI, nodal status, grade and tumor size

Nielsen, et al 2011
### MA5 Results: OS of basal vs. HER-2 vs. all others

#### Question of Anthracycline vs no Anthracycline

<table>
<thead>
<tr>
<th>Biological Subtype And Treatments</th>
<th>N</th>
<th>5-Year OS (95% CI)</th>
<th>P-value</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Log-rank Wilcoxon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMF Core Basal</td>
<td>35</td>
<td>71% (56 - 86)</td>
<td>&lt;0.0001</td>
<td>0.90 (0.50 - 1.60)</td>
</tr>
<tr>
<td>HER2</td>
<td>50</td>
<td>38% (24 - 51)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>All Others</td>
<td>182</td>
<td>80% (73 - 85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEF Core Basal</td>
<td>35</td>
<td>51% (35 - 68)</td>
<td>0.04</td>
<td>1.84 (1.09 – 3.11)</td>
</tr>
<tr>
<td>HER2</td>
<td>63</td>
<td>63% (47 - 76)</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>All Others</td>
<td>168</td>
<td>84% (78 - 89)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Interaction test, basal status vs. treatment response: p = 0.06*
<table>
<thead>
<tr>
<th></th>
<th>CEF</th>
<th>CMF</th>
<th>Relative Risk (CI; CEF:CMF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2 IHC/FISH (+)</td>
<td>44</td>
<td>47</td>
<td>0.55 [0.31–0.80]</td>
</tr>
<tr>
<td>HER2 IHC/FISH (−)</td>
<td>177</td>
<td>186</td>
<td>1.01 [0.63–1.40]</td>
</tr>
<tr>
<td>p=0.0538</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triple negative</td>
<td>44</td>
<td>55</td>
<td>1.57 [0.55–2.59]</td>
</tr>
<tr>
<td>Non-Triple Negative</td>
<td>200</td>
<td>212</td>
<td>0.70 [0.23–1.17]</td>
</tr>
<tr>
<td>p=0.156</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Core Basal (IHC)</td>
<td>35</td>
<td>35</td>
<td>1.82 [0.76–2.88]</td>
</tr>
<tr>
<td>Non Core Basal</td>
<td>209</td>
<td>232</td>
<td>0.71 [0.22–1.20]</td>
</tr>
<tr>
<td>p=0.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aBC(+)</td>
<td>33</td>
<td>39</td>
<td>1.87 [0.87–2.87]</td>
</tr>
<tr>
<td>aBC(−)</td>
<td>132</td>
<td>136</td>
<td>0.63 [0.38–0.88]</td>
</tr>
<tr>
<td>p=0.00339</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal (PAM50)</td>
<td>45</td>
<td>49</td>
<td>1.32 [0.71–1.94]</td>
</tr>
<tr>
<td>Non Basal (PAM50)</td>
<td>176</td>
<td>185</td>
<td>0.68 [0.42–0.93]</td>
</tr>
<tr>
<td>p=0.0392</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 2: predictive biomarkers of CEF vs CMF on NCICCTG MA.5**

Relative risks and standard error of relative risks were calculated using the approximation method described at [http://www.stat.cmu.edu/~hseltman/files/ratio.pdf](http://www.stat.cmu.edu/~hseltman/files/ratio.pdf). Interaction test of relative risks were calculated using the method described at [http://www.hutchon.net/CompareRR.htm](http://www.hutchon.net/CompareRR.htm)
# Bevacizumab in Triple Negative

<table>
<thead>
<tr>
<th>Regimen</th>
<th>DFS HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECOG 2100</td>
<td>Weekly paclitaxel + bevacizumab 0.53 (0.41-0.70)</td>
</tr>
<tr>
<td>AVADO</td>
<td>Docetaxel + bevacizumab 0.68 (NR~1.00)</td>
</tr>
<tr>
<td>RIBBON-1</td>
<td>Chemotherapy + bevacizumab 0.72 (0.49-1.06)</td>
</tr>
<tr>
<td><strong>OS HR (95% CI)</strong></td>
<td>Meta-analysis 3 first-line studies chemo + bevacizumab 0.96 (0.79-1.16)</td>
</tr>
</tbody>
</table>

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**GEPARQUINTO HER2-Neg**

Von Minckwitz, SABCS 2010, ASCO 2011

**Odds ratio of benefit of Bevacizumab added to EC-Doc:**

- **Overall**: OR 1.21
- **ER/PgR negative**: OR 1.42
- **ER/PgR positive**: OR 1.05
- **T1-3 and N0-2**: OR 1.17
- **T4 or N3**: OR 1.70

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E: Epirubicin 90 mg/m²
C: Cyclophosphamide 600 mg/m²
Be: Bevacizumab 15 mg/kg
(3 weeks cycles)

Doc: Docetaxel 100mg/m²
Implications for Treatment

- Chemotherapy remains the mainstay for TNBC but we need to start to classify TNBC correctly into basal subtypes and others
- Which chemotherapy?
- For the BRCA+ evidence of cisplatin responsiveness
- Other targets are being assessed
  - PARP, MET, MEK, EZHZ,
  - Angiogenesis and Hypoxia signatures
  - Androgen receptor
  - Etc
Adjuvant Impact of Trastuzumab

N9831/NSABP B31 Joint Analysis at 4 Years:
- 48% improved DFS
- 39% improved OS

BCIRG 006 at 5 years:
- 36% improved DFS with AC-TH
- 24% improved DFS with TCH

Perez EA et al, JCO 2011; Slamon D et al, NEJM 2011

HER2-targeting converts a high risk tumor type to moderate risk. How do we get better?
- Lapatinib? Pertuzumab? TDM1?
- Other novel HER2- or other pathway-targeting agents?
- Dual vs single?
- Or do we learn how to classify HER2 + better
1. Took prototypical samples and subtype assignments from microarray
2. Used qRT-PCR data for 160 genes
3. Selected optimal gene subset for subtype classifications using 10-fold CV
4. Identified 50 optimal genes, and samples, giving a robust training set (PAM50)
PAM50 subtype within clinically HER2+

Is there variable response to HER2-targeting in any subset – not identified

from Cheang M et al, SABCS 2011
ER and clinHER2 status breakdown within PAM50 HER2-E tumors

NCIC MA.5 (N=103)  NCIC MA.12 (N=80)  Combined microarray dataset\(^1\) (N=55)

- \(\text{clinHER2++; ER-}\): 14\%  15\%  18\%
- \(\text{clinHER2++; ER+}\): 24\%  26\%  15\%
- \(\text{clinHER2--; ER-}\): 43\%  35\%  51\%
- \(\text{clinHER2--; ER+}\): 19\%  24\%  16\%

\(^1\text{Prat & Perou Mol Oncol. 2011}\)
Trastuzumab + docetaxel/capecitabine response according to PAM50 subtype and ER status within clinHER2+ (n=27)

<table>
<thead>
<tr>
<th>Classification</th>
<th>pCR rate</th>
<th># of patients</th>
<th>OR* (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ClinHER2+</td>
<td>10 (37%)</td>
<td>27</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PAM50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal A</td>
<td>1 (50%)</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal B</td>
<td>0 (0%)</td>
<td>7</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Normal-like</td>
<td>2 (67%)</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal-like</td>
<td>0 (0%)</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2-E</td>
<td>7 (58%)</td>
<td>12</td>
<td>8 (1.1-50.9)</td>
<td>0.036</td>
</tr>
<tr>
<td>ER</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER+</td>
<td>2 (17%)</td>
<td>12</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>ER-</td>
<td>8 (53%)</td>
<td>15</td>
<td>6 (0.9-36.3)</td>
<td>0.062</td>
</tr>
</tbody>
</table>

*Adjusted for T stage at baseline

Cheang et al SABCS 2011
Anthracycline/taxane-based neoadjuvant response according to PAM50 subtype and ER status within clinHER2+ (n=67)

<table>
<thead>
<tr>
<th>Classification</th>
<th>pCR rate</th>
<th># of patients</th>
<th>MVA* OR (95% C.I.)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ClinHER2+</td>
<td>26 (39%)</td>
<td>67</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Luminal A</td>
<td>0 (0%)</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal B</td>
<td>1 (14%)</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Normal-like</td>
<td>1 (20%)</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2-E</td>
<td>17 (49%)</td>
<td>35</td>
<td>14 (1.1-172)</td>
<td>0.039</td>
</tr>
<tr>
<td>Basal-like</td>
<td>7 (54%)</td>
<td>13</td>
<td>11 (0.8-163)</td>
<td>0.076</td>
</tr>
<tr>
<td>ER+</td>
<td>4 (14%)</td>
<td>28</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>ER-</td>
<td>22 (56%)</td>
<td>39</td>
<td>10 (1.4-73)</td>
<td>0.021</td>
</tr>
</tbody>
</table>

*MVA model included grade, clinical T stage and nodal status measured at baseline

Cheang et al, SABCS 2011
Why Is this Relevant?

- Increasing evidence of the differential response of different tumour types to treatments
- Clearly differences in sensitivity/ response
- Clearly differences in sensitivity to specific targeted drugs – ER/HER2
- To understand resistance and response we need to have cleaner cohorts
- To effectively develop new agents we need cleaner cohorts
Somatic and germline variation in the genome/epigenome underpins tumour development – however it occurs at every scale.

Eg. BRCA2, p53, BRAF, etc

Single base error/mutation

…ATTTGATCCGGG…

↓

…ATTTGAACCGGG…

Until very recently it has been impractical to comprehensively establish genome variation at all scales.
Metabric
(Molecular Taxonomy of Breast Cancer International Consortium)

- SNP6.0 arrays and Illumina bead expression arrays (frozen tissues) from 2167 primary breast cancers where > median 10 years clinical outcome data was available.
- 1000 case discovery set, 1000 case validation set
Joint clustering of copy number and expression reveals ~10 discrete stable groupings within 1000 breast cancer patients.

- **CNA frequency**
- Chi-square P-value (subtype test)

1. **non HER2, 17q subtype**
2. **HER2 subtype**
3. **predominantly basal TN**
4. **genomically quiescent tumours (17% of breast cancers)**
5. **novel 11q subtype**
6. **1q, mostly quiescent tumours**
Relationship between integrative cluster groups and breast cancer specific survival over 15 years
Challenge To Translational Research

• We need to validate these subtypes as both having both prognostic and predictive value
• We need to bring into clinical research technologies which can define cleaner cohorts and develop new agents with these populations
• Along side we need to begin to understand the evolution of cancers to tackle resistance
• Plus we need to understand the host and not just the tumour
Summary

• Breast cancer subtype influences risk of relapse, local and distant, site of relapse and response to therapy

• Retrospective analysis in luminal subtypes
  – Response to hormones
  – Response to the addition of chemotherapy
  – Response to taxanes, luminal B retrospective data
Summary

• In the HER2
  – Response to antiHER2 and specific chemo is better when the subtype is more specifically defined

• In the TNBC
  – Differences in BRCA+ vs BRCA-
  – Subtypes of TNBC must be assessed and in particular the basal like vs the others

• New classifications may further delineate responses to specific targeted and non targeted therapy

• Choice of therapy at this time must be carefully assessed as we do not yet have prospective data

• Trials need to collect tissue and understand the heterogeneity more completely
All Breast Cancer

- ER+ 65-75%
- HER2+ 15-20%
- Basaloid ~9%
- BRCA like ~6%