Special Symposium: Clinical Relevance of Genotyping ER Positive Breast Cancer
Emerging Treatment Options in the Genomic Era

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Suntec City, Singapore
19th December, 2015
Disclosures

• Janice Tsang

• Consultant or Advisory Role: AstraZeneca, Eisai, GlaxoSmithKline, Novartis & Pfizer
Changing Portraits of Breast Cancer
Background

• Results of our translational research in the last decade, have revolutionized our understanding of breast cancer as a heterogeneous disease:

• Genomics and transcriptomic have been helping to narrow down the number of candidate oncogenes - “One-size-fits-all” approach becomes less relevant
### Genomic Medicine for Breast Cancer Patients (beyond ER, PR, HER2, and chemotherapy)

<table>
<thead>
<tr>
<th>Altered genes with predictive biomarker potential</th>
<th>Treatment approach</th>
<th>Strength of hypothesis for somatic alteration–targeted drug match (reference)</th>
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</thead>
<tbody>
<tr>
<td><strong>PIK3CA mutation</strong></td>
<td>PIK3CA-selective inhibitors</td>
<td>2 Phase I BYL719 (18)</td>
</tr>
<tr>
<td><strong>FGFR1 amplification, FGF3 amplification, other FGF ligands and receptors, and rare receptor mutations</strong></td>
<td>FGFR small-molecule inhibitors and antibodies</td>
<td>2 Phase I BGJ398 (48) and phase I E3800 (47)</td>
</tr>
<tr>
<td><strong>Inherited and somatic BRCA1 and BRCA2 mutation</strong></td>
<td>PARP inhibitors</td>
<td>2 Olaparib (49) and veliparib: NCT01506609</td>
</tr>
<tr>
<td><strong>Cyclin D1/CDK4/CDK6 amplification or deletion of CDKN1B, CDKN2A, and CDKN2B</strong></td>
<td>CDK4/6 inhibitors</td>
<td>2 PD0332991 (40)</td>
</tr>
<tr>
<td><strong>AKT1–3 gain-of-function mutation/gene fusion via translocation/amplification</strong></td>
<td>AKT inhibitors</td>
<td>3 MK-2206: NCT01277757</td>
</tr>
<tr>
<td><strong>GATA3 mutation</strong></td>
<td>Aromatase inhibition</td>
<td>3 Retrospective analysis of Z1031 (4)</td>
</tr>
<tr>
<td><strong>PTEN/INPP4B loss-of-function mutation/deletion/loss of expression in TNBC</strong></td>
<td>Broad-spectrum PI3K pathway inhibitors</td>
<td>3 BKM120: NCT01629615</td>
</tr>
<tr>
<td><strong>MDM2 amplification in TP53 wild-type tumors</strong></td>
<td>MDM2 inhibitors</td>
<td>3 RO5503781: NCT01462175</td>
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<tr>
<td><strong>HER2 mutation</strong></td>
<td>Small-molecule HER2 kinase inhibitors</td>
<td>3 Neratinib: (NCT01670877)</td>
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<tr>
<td><strong>PIK3R1 loss-of-function mutation</strong></td>
<td>PI3K pathway inhibitors?</td>
<td>4</td>
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<td><strong>MLL family member mutation</strong></td>
<td>HDAC inhibition?</td>
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<tr>
<td><strong>Rare RTK mutations</strong></td>
<td>Various matched inhibitors?</td>
<td>4</td>
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</table>

NOTE: Number 1 indicates approved therapy; 2, early evidence of efficacy; 3, clinical investigations under way; and 4, clinical investigations not yet activated.

Ellis and Perou, Cancer Discovery, 2013 (PMID 23319768)
Development of Genomic Signatures...

• **Discovery**
  - NGS, RNA seq, proteomics
  - Analytical validation
  - Training sets and validation sets

• **Clinical Validation**
  - Prognostic & Predictive value
  - Retrospective vs Prospective L-T FU
  - Compared with old therapies

• **Clinical Utility**
Best practices for translational omics studies Institute of Medicine (www.iom.edu/translationalomics)
Normal Breast

Claudin-low
Basal-like

Luminal A and B

HER2-enriched

Intrinsic Subtypes
Perou et al., Nature 2000
Sorlie et al., PNAS 2001
Sorlie et al., PNAS 2003
Nielsen et al., CCR 2004
Cheang et al., CCR 2008
Parker et al., JCO, Feb 2009
Cheang et al., JNCI 2009
Prat et al., BCR 2010
Nielsen et al., CCR 2010
Cheang et al., CCR 2012

Relapse-free survival

Log Rank p=1.9e-07

Months
Comprehensive molecular portraits of human breast tumours

Diverse mutations of breast cancer subtypes

The Cancer Genome Atlas Network

Predicted somatic non-silent mutations

<table>
<thead>
<tr>
<th>Subtype</th>
<th>PIK3CA</th>
<th>MAP3K1</th>
<th>MAP2K4</th>
<th>GATA3</th>
<th>ML3</th>
<th>CCDC1</th>
<th>AKT1</th>
<th>AKT2</th>
<th>PIK3R1</th>
<th>PIK3R2</th>
<th>TP53</th>
<th>FOXA1</th>
<th>SRB1</th>
<th>RB1</th>
<th>AIP</th>
<th>NF1</th>
<th>PTEN</th>
<th>NTRK2</th>
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<tr>
<td>Luminal A</td>
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<td>Luminal B</td>
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<td>HER2-enriched</td>
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<td>Basal-like</td>
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</table>

Percentages of cases with mutation by expression subtype

- Luminal A: 45% Luminal B: 29% HER2-enriched: 39% Basal-like: 9%
- Luminal A: 12% Luminal B: 29% HER2-enriched: 72% Basal-like: 60%

Clinical data

Copy number status

Mutations per Mb

TCGA Nature 2012
Prognostic Marker

– valuable if provides extra information beyond that provided by clinicopathological features

• A **prognostic** marker
  – associated with clinical outcome irrespective of treatment given
    • tumour size, tumour grade, no. of positive lymph nodes

Predictive Marker

- Predicts clinical benefit from a specific therapy
  - ER – endocrine therapy
  - HER2/neu over-expression – anti-HER2 directed therapies
- KRAS mutation for EGFR therapy
- Pathological complete response (pCR) to predict long-term survival – FDA program
- Some predictive markers also prognostic

BIOMARKERS

**Predictive factors** to select patients for specific therapies

**Prognostic factors** to segregate patients into risk groups

**Surrogate endpoints** as measurements before, during and after treatment, used as a measure of disease activity to determine whether the treatment is working

**Usual steps**
1. Develop a “locked down” version of genomic classifier to identify patients likely to benefit
2. Determine the reproducibility/robust of measurement of the classifier/biomarker
3. With a pre-defined analysis plan using the completely specified classifier/biomarker to design and/or analyze a new clinical trial to test the effectiveness
FDA Definition of “Biomarker”

- A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention

**Use of biomarkers**
- Diagnosis
- Tool for staging disease
- Indicator of disease status
- Predict and/or monitor clinical response to an intervention
Overview of gene expression analysis of human breast tumors


Courtesy of Maggie Cheang
Various Genomic Platforms

1) Immunohistochemical staining (IHC4)
2) Molecular Classification
3) Genomic Expression Profiling Prognostic platform
4) Genomic Expression Profiling Immunomodulatory
5) Targeted sequencing
6) Whole exome (genome) sequencing
7) Big Data

- The Cancer Genome Atlas (TCGA)
  Gene expression, Exome Sequencing, DNA copy number,
  miRNA expression, DNA methylation etc.
- Molecular Taxonomy of Breast Cancer
  International Consortium (METABRIC)

Cuzick et al. J Clin Oncol 2011
A Multi-gene Assay to Predict Recurrence of Tamoxifen-Treated Node-Negative Breast Cancer.


**Figure 1. Panel of 21 Genes and the Recurrence-Score Algorithm.**

The recurrence score on a scale from 0 to 100 is derived from the reference-normalized expression measurements in four steps. First, expression for each gene is normalized relative to the expression of the five reference genes (ACTB [the gene encoding β-actin], GAPDH, GUS, RPLPO, and TFRC). Reference-normalized expression measurements range from 0 to 15, with a 1-unit increase reflecting approximately a doubling of RNA. Genes are grouped on the basis of function, correlated expression, or both. Second, the GRB7, ER, proliferation, and invasion group scores are calculated from individual gene-expression measurements, as follows: GRB7 group score = 0.9 × GRB7 + 0.1 × HER2 (if the result is less than 8, then the GRB7 group score is considered 8); ER group score = (0.8 × ER + 1.2 × PGR + BCL2 + SCUBE2) × 4; proliferation group score = Survivin + Ki67 + MYBL2 + CCNB1 [the gene encoding cyclin B1] + STK15 + 5 (if the result is less than 6.5, then the proliferation group score is considered 6.5); and invasion group score = (CTSL2 [the gene encoding cathepsin L2] + MMP11 [the gene encoding stromelysin 3]) × 2. The unscaled recurrence score (RSU) is calculated with the use of coefficients that are predefined on the basis of regression analysis of gene expression and recurrence in the three training studies**: **RSU = 0.47 × GRB7 group score + 0.34 × ER group score + 1.04 × proliferation group score + 0.10 × invasion group score + 0.05 × CD68 + 0.08 × GSTM1 − 0.07 × BAG1. A plus sign indicates that increased expression is associated with an increased risk of recurrence, and a minus sign indicates that increased expression is associated with a decreased risk of recurrence. Fourth, the recurrence score (RS) is rescaled from the unscaled recurrence score, as follows: RS = 0 if RSU < 0; RS = 20 × (RSU − 6.7) if 0 ≤ RSU ≤ 100; and RS = 100 if RSU > 100.

**Figure 2. Likelihood of Distant Recurrence, According to Recurrence-Score Categories.**

A low risk was defined as a recurrence score of less than 18, an intermediate risk as a score of 18 or higher but less than 31, and a high risk as a score of 31 or higher. There were 28 recurrences in the low-risk group, 25 in the intermediate-risk group, and 56 in the high-risk group. The difference among the groups is significant (P<0.001).
## Gene Expression-based Clinical Assays 2013

<table>
<thead>
<tr>
<th>Test</th>
<th>Approval or Endorsement</th>
<th>Predicting Prognosis</th>
<th>Predicting Treatment Benefit using Randomized Clinical Trials</th>
<th>Randomized Prospective Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OncotypeDX® RT-PCR, FFPE</strong></td>
<td>NCCN: Prediction of chemotherapy benefit</td>
<td>NSABP-B14</td>
<td>NSABP-B20 (+/- CMF) SWOG8814 (+/- CAF)</td>
<td>NSABP-B28 (failed to predict a benefit)</td>
</tr>
<tr>
<td><strong>Prosigna® nCounter, FFPE</strong></td>
<td>CE Mark, FDA: Prediction of 10-year DRFS in ER+, node 0-3, postmenop. treated with endocrine therapy</td>
<td>ATAC, ABCSG08</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td><strong>PAM50 research-based assay RT-PCR and microarray, FFPE and fresh</strong></td>
<td>NA</td>
<td>NCIC-MA5, NCIC-MA12, GEICAM9905, multiple non-randomized trial cohorts</td>
<td>NO</td>
<td>NCIC-MA12</td>
</tr>
<tr>
<td><strong>MammaPrint® microarray, fresh and FFPE</strong></td>
<td>FDA (fresh): risk for distant mets, &lt; 0.1 years, Stage I-II, tumor ≤ 3cm and node-negative</td>
<td>multiple non-randomized trial cohorts including RASTER</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td><strong>EndoPredict® RT-PCR, FFPE</strong></td>
<td>CE Mark</td>
<td>ABCSG06, ABCSG08, GEICAM9906</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td><strong>Breast Cancer Index SM RT-PCR, FFPE</strong></td>
<td>NO</td>
<td>ATAC, Stockholm, multiple non-randomized trial cohorts</td>
<td>NO</td>
<td>NO</td>
</tr>
</tbody>
</table>

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Relative changes in breast tumour size measured by 3-dimensional ultrasound in BC patients receiving neoadjuvant letrozole...
A 4-gene predictive model to clinical response to AI by 2 wks is associated with clinical response

The molecular response to letrozole was characterized and a four-gene classifier of clinical response was established (accuracy of 96%) on the basis of the level of two genes before treatment (one gene [IL6ST] was associated with immune signaling, and the other [NGFRAP1] was associated with apoptosis) and the level of two proliferation genes (ASPM, MCM4) after 2 weeks of therapy. The four-gene signature was found to be 91% accurate in a blinded, completely independent validation data set of patients treated with anastrozole.

Deregulated immune and apoptotic responses before treatment and cell proliferation that is not reduced 2 weeks after initiation of treatment are functional characteristics of breast tumors that do not respond to AIs.

Whole Genome Analysis Informs Breast Cancer Response to Aromatase Inhibition


Discovery Set

Pathway signatures connections between mutations and clinical outcomes...low risk PEPI score cluster with luminal A subtype...

Genomic wide somatic mutations (WGA) and response to neoadj AI

Ki-67 <= 10%

Ki-67 >10%
Difficult to identify the key mutation pathway in luminal BC—due to the inter-connectedness of the complicated network with too many ways to perturb a pathway...
A genomic index for sensitivity to endocrine therapy (SET) index was defined from genes coexpressed with ESR1 in 437 microarray profiles from newly diagnosed breast cancer, unrelated to treatment or outcome. The association of SET index and ESR1 levels with distant relapse risk was evaluated from microarrays of ER-positive breast cancer in two cohorts who received 5 years of tamoxifen alone as adjuvant endocrine therapy (n = 225 and 298, respectively).

The SET index of ER-related transcription predicted survival benefit from adjuvant endocrine therapy, not inherent prognosis. Prior chemotherapy seemed to enhance the efficacy of adjuvant endocrine therapy related to SET index.
Added Value of Precision Medicine in the Genomic Era

- “One-size” does not fit all
- Identifying the right therapy or the right patient
  - Enhance clinical outcomes
  - Increase benefit : risk ratio
  - Accelerate new therapeutic development for breast cancer
Added Value of Precision Medicine in the Genomic Era

- New targets = new biomarkers
- Efficient development of validated companion diagnostic markers essential
- Translational studies important to better understand reasons for success and failure, and to gain new insights in breast cancer biology that may provide new therapeutic opportunities
Emerging Treatment Options vs Challenges...
Cancers not limited to BC are highly dynamic evolutionary...

Ongoing linear and branching evolution results in multiple simultaneous subclones that may individually be capable of giving rise to episodes of disease relapse and metastasis. The dynamic clonal architecture is shaped by mutation and competition between subclones in light of environmental selection pressures, including those that are exerted by cancer treatments.

![Figure 1. The evolution of clonal populations](image-url)
From a single common disease to many rare diseases

- **Intratumoral heterogeneity**
  - How many populations? Hierarchy?
  - Primary vs relapsed/metastatic tumour samples
  - Solid biopsy vs liquid biopsy (CTCs, plasma DNA)

- **Intertumoral heterogeneity**
  - How many tumours?
  - Molecular segmentation or granularity?

- **Heterogeneity of the host**
  - Immunity

_Heterogeneity of Breast Cancer_
Heterogeneity of Breast Cancer

Table 4 | Clinical implications of tumour heterogeneity in breast cancer

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

Heterogeneity of Breast Cancer

- Tumour heterogeneity in breast cancer even occurs at single cell level

Change of ER/PgR & HER2 status

• 3-28% of all metastatic lesions will either loose or acquire ER expression.
• 3-25% of the patients will loose or acquire the HER2 overexpression or amplification.

J Natl Cancer Inst 93: 1441-6, 2001
Br J Cancer 93:552-6, 2005
Cancer 103: 1763-9, 2005
Overcoming Endocrine Resistance:
Mode of Action in HER2+/HR+ Breast Cancer – Cross-talk between the ER and HER-2 pathways...

Further New Challenges...

• The incidence of breast cancer is increasing
• The breast cancer patients are living longer
• Our research and clinical trials have proven success...
• Matching science with the affordability...
  ...the high expectation of the patient and the general public...

patient classification, and selection for specific therapies
Proteomics in Clinical Trials

Basket Trials
- aim to test one drug or one particular genetic mutation across multiple organs.

Umbrella Trials
- seek to test a drug or drugs across multiple genetic mutations within a particular type of cancer. For example, the I SPY-2 umbrella trial in breast cancer.

One of the major uses of genomics in clinical research is in the design and execution of novel clinical trials. Two such types of trials are basket and umbrella trials. In the basket trial depicted here, one drug is being tested against a particular genetic mutation (green dots) across liver, lung, bone, colon, and stomach cancers. In the umbrella trial illustrated here, three different drugs are being tested against multiple genetic mutations (yellow, green, blue, and red dots) within lung cancer.

“Transforming lives through research”, AACR (2014)
Comparative genomic hybridisation array and DNA sequencing to direct treatment of metastatic breast cancer: a multicentre, prospective trial (SAFIR01/UNICANCER)

Fabrice André, Thomas Bachelot, Frederic Commo, Mario Campone, Monica Arnedos, Véronique Dieras, Magali Lacroix-Triki, Ludovic Lacroix, Pascale Cohen, David Gentien, Jose Adélaide, Florence Dalenc, Anthony Goncalves, Christelle Levy, Jean-Marc Ferrero, Jacques Bonneterre, Claudia Lefevre, Marta Jimenez, Thomas Filleron, Hervé Bonnefoi

Summary
Background Breast cancer is characterised by genomic alterations. We did a multicentre molecular screening study to identify abnormalities in individual patients with the aim of pro genomic alterations.

Methods From June 16, 2011, to July 30, 2012, we recruited patients for biopsy in 18 centres in France. Comparative genomic hybridisation PIK3CA (exon 10 and 21) and AKT1 (exon 4) were used to assess metastases were decided on the basis of identified genomic alteration.

SAFIR01: Study Flow

- Biopsy metastases in patients PR/SD under treatment
- 2 Frozen samples
- 1 FFPE sample
- Targeted therapy according to the genomic profile at the time of PD
- Identification of a targetable Genomic Alteration by a multicentric multidisciplinary team
- Whole genome CGH array (gene copy numbers)
- Sanger sequencing hot spots PIK3CA/AKT1
The UK Molecular profiling of Advanced breast cancer to inform Therapeutic Choices (MATCH study)

Clinical Leads: Dr Nicholas Turner (Royal Marsden) & Dr Alistair Ring (Brighton and Sussex)

Molecular Sequencing Lead: Dr David Gonzalez de Castro (Institute of Cancer Research / Royal Marsden)

Methodology Lead: Prof Judith Bliss (ICR-CTSU)

Proposed funders: applications under review by Breakthrough Breast Cancer & Cancer Research UK

Proposed pharmaceutical partners: AstraZeneca

Treatment until disease progression with therapeutic targeted against:
- HER2 mutation (2% incidence)
- EGFR amplification (3% incidence)
- AKT1 mutation (3% incidence)
- AKT activation basket (3-4% incidence)
- Sporadic HR basket (4% incidence)
- FGFR activation basket (4% incidence)
- Future cohorts - ESR1 or ERBB3 mutation (15% incidence)
Conclusion

- We have entered the **genomic era** where we are one step forward to further enhancement of personalized medicine and **precision medicine**.

- **Clinical validity** is demonstrated yet awaiting the prime time for clinical utility with demonstration of clinically meaningful benefit.

- Basket trial or umbrella trial should be the trend with **prospective L-T FU** with mutational analysis.
Conclusion

• There are emerging new technologies with liquid biopsy (CTCs, ctDNA), leading to potential serial and non-invasive mutational analyses, likely to become available in the near future.

• Efforts to realize the dream of personalized treatment for breast cancer will include drug development and intelligent design of clinical trials for increasingly small subgroup of patients with specific host and disease characteristics.
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