Integration of molecular analysis in the management of metastatic breast cancer

Dr Nicholas Turner

ESMO 2014
Disclosure slide

• Advisory Board – Genomic health, Pfizer
Outline

• Genetic analysis of metastatic breast cancer
• Prognostication in metastatic breast cancer
• Prediction of sensitivity to treatment
Comprehensive molecular portraits of human breast tumours

Diverse mutations of breast cancer subtypes

The Cancer Genome Atlas Network*
Why do we need genetic analysis of recurrent breast cancer?
HER2 amplification - discordance with primary

Estimates with 95% confidence intervals

<table>
<thead>
<tr>
<th>Study</th>
<th>Publication Year</th>
<th>Test</th>
<th>D/N*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodes only</td>
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<td></td>
<td></td>
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<tr>
<td>Tsutsui</td>
<td>2002</td>
<td>IHC</td>
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<td>Carlsson</td>
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<td>Santiago</td>
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<td>Cardoso</td>
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<td>Azam</td>
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<td>5/100</td>
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<td>Santinelli (nodes)</td>
<td>2008</td>
<td>I/F</td>
<td>3/54</td>
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<td>Simon</td>
<td>2001</td>
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<td>Aoyama</td>
<td>2010</td>
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<td>3/33</td>
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<td>Nodes or Local Recurrence or Distant Metastases</td>
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<td>2000</td>
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<td>Sekidō</td>
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<td>Distant Metastases or Local Recurrence#</td>
<td></td>
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<tr>
<td>Idrissinghe</td>
<td>2010</td>
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<tr>
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<td>Distant Metastases only</td>
<td></td>
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<td>Tapia</td>
<td>2007</td>
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<td>Vincent-Salomon</td>
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<td>IHC</td>
<td>2/44</td>
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<tr>
<td>Gancberg</td>
<td>2002</td>
<td>IHC</td>
<td>6/100</td>
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<tr>
<td>Simmons</td>
<td>2009</td>
<td>FISH</td>
<td>2/25</td>
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<tr>
<td>Regitinig</td>
<td>2004</td>
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</tr>
<tr>
<td>Lower</td>
<td>2009</td>
<td>IHC</td>
<td>12/382</td>
</tr>
</tbody>
</table>

Houssami et al BCRT 2011
2500 cancers from BCIRG studies

Central re-testing of HER2 by FISH

Table 2. DAKO HercepTest immunohistochemical assay results from outside laboratories compared with FISH assay results from the BCIRG central laboratories

<table>
<thead>
<tr>
<th></th>
<th>Outside laboratory immunohistochemistry scores (0-3+)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>FISH negative (%)</td>
<td>296 (96.4)</td>
<td>142 (94.7)</td>
</tr>
<tr>
<td>FISH positive (%)</td>
<td>11 (3.6)</td>
<td>8 (5.3)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>307 (100)</td>
<td>150 (100)</td>
</tr>
</tbody>
</table>
HER2 - Discordance with primary breast cancer

HER2 discordant in ~5% of metastatic breast cancer compared to primary breast cancer

Standard of care to repeat biopsy on recurrence where feasible.
Genetic events are acquired in metastatic breast cancer

ESR1 ligand-binding domain mutations in hormone-resistant breast cancer

Weiyi Toy¹, Yang Shen², Helen Won³, Bradley Green³, Rita A Sakr⁴, Marie Wilt⁵, Zhiqiang Li³, Kinisha Gala¹, Sean Fanning³, Tari A King³, Clifford Hudis⁵,⁶, David Chen⁷, Tetiana Taran⁷, Gabriel Hortobagyi⁸, Geoffrey Greene³, Michael Berger¹,⁹, José Baselga¹,⁵ & Sarat Chandrarapaty¹,⁵,⁶

ESR1 mutations occur in 20% of endocrine resistant resistant ER positive breast cancer

Toy et al Nat Genetics 2013
**ESR1 mutations active the estrogen Receptor**

Mutations in ligand binding domain activate ER signaling

Cause resistance to Ais

Potentially sensitive to SERDs
Single cell sequencing in primary breast cancer

ER positive BC

TNBC

Wand et al Nature 2014
Genetic heterogeneity and drug resistance

Genetic heterogeneity as the engine of targeted therapy resistance

Turner et al Lancet Oncol 2012
Whole exome sequencing (WES) of HER2+ metastatic breast cancer from patients with or without prior trastuzumab exposure: A correlative analysis of TBCRC003


on behalf of the Translational Breast Cancer Research Consortium

2014 ASCO Annual Meeting
Breast Cancer - Her2/ER - Poster Highlights Session
HER2 mutations have previously been identified in ~2% of HER2- cancers and <1% of primary HER2+ cancers (CBio portal).

In 3/40 patients who had received prior trastuzumab (8%), we identified a HER2 L755S kinase mutation.

HER2 L755S results in resistance to lapatinib and sensitivity to irreversible inhibitors (e.g. neratinib).

5 additional patients, 3 of whom received prior trastuzumab, had uncharacterized mutations in HER2 at low allelic fractions, including a novel kinase domain mutation D742N in a patient with prior trastuzumab.

Functional characterization of novel HER2 mutations is currently underway.

Kancha et al, PLOS One, 2011; Bose et al, Cancer Discovery, 2013.
How do we sample the genetics of metastatic breast cancer?
ARRAY CGH AND DNA SEQUENCING TO PERSONALIZE THERAPY FOR METASTATIC BREAST CANCER: A PROSPECTIVE NATIONAL TRIAL (UNICANCER SAFIR-01)

Personalized Medicine: To identify and target the right molecular pathway for each patient

Targeted therapy according to the genomic profile

Biopsy metastases

Whole genome profiling

Identification of the Genomic Alteration
SAFIR01: Targetable genomic alterations in patients treated and response to therapy

Response rate numerically higher than expected (30%)
Her2-negative metastatic breast cancer no more than 1 line chemotherapy

Biopsy metastatic site: Next generation sequencing Array CGH

Target defined by 1st generation Virtual cell (CCLE)

Chemotherapy 6-8 cycles

No alteration

No PD

Followed up but not included

8 Targeted therapies according to 51 Molecular alterations

SOC

R
Newly diagnosed or 1st Line MBC Patients

N=1,300

Screening Failure n=300

‘Actionable’ Mutation(s) (n=300)

Downstream Targeted Clinical Trials as first or second line

‘Non-Actionable’ Mutations (n=700)

Standard of Care

Clinical Outliers (Exceptional Responders and Rapid Progressors) to be subjected to WES
How do we sample the genetics of metastatic breast cancer?

Non-invasive detection
Non-invasive interrogation of cancer genetics

Crowley et al Nat Rev Clin Oncol 2013
# Androgen receptor amplification in CTC

**AR FISH**

<table>
<thead>
<tr>
<th>Panel d</th>
<th>Patient 71</th>
</tr>
</thead>
<tbody>
<tr>
<td>Event 73</td>
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</tr>
<tr>
<td>Event 106</td>
<td><img src="image2.png" alt="Image" /></td>
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<td>Event 423</td>
<td><img src="image3.png" alt="Image" /></td>
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<tr>
<td>Event 725</td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>

*Attard et al, Cancer Res 2009*
HER2 status in plasma DNA – independent validation set

**(A)**

- **Sensitivity vs. 1 - Specificity** graph.

**(B)**

- Graph showing informative droplet ratio versus number of informative droplets.

**(C)**

- **Tumour HER2 status** table:
  - **Amp**
    - +ve: 7
    - -ve: 4
  - **Non-Amp**
    - +ve: 3
    - -ve: 44

- **Sensitivity**: 64%
- **Specificity**: 94%
- **PPV**: 70%
- **NPV**: 92%

Gevensleben et al. *Clin Cancer Res* 2013
Detection of resistance mutations in plasma

Metastatic colon cancer on cetuxumab

Diaz et al Nature 2012
Genetic events are acquired in metastatic breast cancer

ESR1 ligand-binding domain mutations in hormone-resistant breast cancer

Weiyi Toy1, Yang Shen2, Helen Won3, Bradley Green3, Rita A Sakr4, Marie Will5, Zhiqiang Li1, Kinisha Gala1, Sean Fanning4, Tari A King4, Clifford Hudis5,6, David Chen7, Tetiana Taran2, Gabriel Hortobagyi8, Geoffrey Greene3, Michael Berger1,9, José Baselga1,5 & Sarat Chandarlapaty1,5,6

Nat Genetics 2013
Prognostication in metastatic breast cancer

Tumour bulk surrogates

Tumour biology
Tumour bulk surrogates – circulating tumour cells

Any line therapy

First line

Cristofani et al NEJM 2004
Cristofani et al JCO 2005
Tumour bulk surrogates – circulating tumour DNA

Level of circulating tumour DNA is prognostic

Quantiles of ctDNA and Overall Survival

Probability of Survival

Days

25% quant. ctDNA (10.35)
50% quant. ctDNA (150.1)
75% quant. ctDNA (710.11)
95% quant. ctDNA (8016.3)

P<0.001

Dawson et al NEJM 2013
Tumour Biology
Breast cancer subtypes – ER positive subtypes

Luminal B
Low ER
High Proliferation

Luminal A
High ER
Low Proliferation
21 gene recurrence score - Oncotype DX

Formalin fixed tumour sections → RNA extraction → RT-PCR to assess gene expression

Sixteen cancer genes and five reference genes from three studies:

**Proliferation group**
- Ki-67
- STK15
- Survivin
- Cyclin B1
- MYBL2

**Estrogen group**
- ER
- PR
- Bcl-2
- SCUBE2

**Invasion group**
- Stromelysin 3
- Cathepsin L2

**HER2 group**
- GRB7
- HER2

**GSTM1**

**BAG1**

**CD68**

**Reference group**
- Beta-actin
- GAPDH
- RPLPO
- GUS
- TFRC

**Calculation of gene recurrence score**

\[ RS = +0.47 \times \text{HER2 group score} -0.34 \times \text{estrogen group score} +1.04 \times \text{proliferation group score} +0.10 \times \text{invasion group score} +0.50 \times \text{CD68} -0.08 \times \text{GSTM1} -0.07 \times \text{BAG1} \]

**Interpretation of recurrence score**

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>RS (0–100)</th>
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<tbody>
<tr>
<td>Low risk</td>
<td>RS &lt; 18</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>RS ≥ 18 and &lt; 31</td>
</tr>
<tr>
<td>High risk</td>
<td>RS ≥ 31</td>
</tr>
</tbody>
</table>
NSABP B14 - Recurrence Score predicts recurrence

675 women treated with tamoxifen only

All ER positive, node negative

Paik et al NEJM 2004
Prognostic impact of 21-gene Recurrence Score® in patients presenting with Stage IV breast cancer


Translational Breast Cancer Research Consortium
TBCRC 013
Samples and Statistical Analyses

- 110 pts (86%) with pre-treatment primary tumor samples suitable for 21-gene Recurrence Score® analysis

- Clinical variables, time to first progression (TTP) and 2yr overall survival (OS) were correlated with 21-gene Recurrence Score® using log-rank, Kaplan-Meier and Cox regression
  - All patients (any ER, Her2)
  - ER positive (IHC)
  - ER positive Her 2 negative (IHC, FISH)
2yr Overall Survival by Risk Group

<table>
<thead>
<tr>
<th>2 yr OS, %</th>
<th>RS&lt;18</th>
<th>RS 18-30</th>
<th>RS &gt;30</th>
<th>Log rank, p</th>
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</thead>
<tbody>
<tr>
<td>All pts (n=102)</td>
<td>100 (78-100)</td>
<td>100 (78-100)</td>
<td>80 (69-93)</td>
<td>0.049</td>
</tr>
<tr>
<td>ER+ (n=86)</td>
<td>100 (78-100)</td>
<td>100 (78-100)</td>
<td>77 (64-94)</td>
<td>0.016</td>
</tr>
<tr>
<td>ER+HER2- (n=70)</td>
<td>100 (78-100)</td>
<td>100 (75-100)</td>
<td>69 (51-93)</td>
<td>0.001</td>
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</table>
ER+ pts treated with 1st line endocrine tx
High RS shorter TTP

ER positive (IHC)

ER pos Her 2 neg

<table>
<thead>
<tr>
<th>Median TTP, mos</th>
<th>RS&lt;18</th>
<th>RS 18-30</th>
<th>RS &gt;30</th>
<th>Log rank, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+ (n=50)</td>
<td>NR (18-NR)</td>
<td>22 (13-NR)</td>
<td>15 (8-NR)</td>
<td>0.009</td>
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<tr>
<td>ER+HER2- (n=49)</td>
<td>NR (18-NR)</td>
<td>22 (13-NR)</td>
<td>15 (8-NR)</td>
<td>0.016</td>
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</table>
ER+ pts treated with 1st line chemotherapy
No difference by RS

<table>
<thead>
<tr>
<th>Median TTP, mos</th>
<th>RS&lt;18</th>
<th>RS18-30</th>
<th>RS&gt;30</th>
<th>Log rank, p</th>
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<tr>
<td>ER+ (n=36)</td>
<td>10.5 (4-NR)</td>
<td>20 (12-NR)</td>
<td>16 (9-NR)</td>
<td>0.54</td>
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<tr>
<td>ER+HER2- (n=21)</td>
<td>10.5 (4-NR)</td>
<td>18 (12-NR)</td>
<td>13 (9-NR)</td>
<td>0.56</td>
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</table>
Is prognostication important?
Abstract CT101

Final Results of a Randomized Phase 2 Study of Palbociclib (PD 0332991) a Cyclin-Dependent Kinase (CDK) 4/6 Inhibitor, in Combination with Letrozole vs Letrozole Alone for First-Line Treatment of ER+, HER2– Advanced Breast Cancer (PALOMA-1/TRIO-18)

RS Finn,1 JP Crown,2 I Lang,3 K Boer,4 IM Bondarenko,5 SO Kulyk,6 J Ettl,7 R Patel,8 T Pinter,9 M Schmidt,10 Y Shparyk,11 AR Thummala,12 NL Voytko,13 X Huang,14 ST Kim,14 S Randolph,14 DJ Slamon1

1University of California Los Angeles, Los Angeles, CA, USA; 2Irish Cooperative Oncology Research Group, Dublin, Ireland; 3Orszagos Onkolgiai Intezet, Budapest, Hungary; 4Szent Margit Korhaz, Onkologia, Budapest, Hungary; 5Dnipropetrovsk City Multiple-Discipline Clinical Hospital, Dnipropetrovsk, Ukraine; 6Municipal Treatment-and-Prophylactic Institution, Donetsk, Ukraine; 7Technical University of Munich, Munich, Germany; 8Comprehensive Blood and Cancer Center, Bakersfield, CA, USA; 9Petz Aladar Megyei Oktato Korhaz, Gyor, Hungary; 10University Hospital Mainz, Mainz, Germany; 11Lviv State Oncologic Regional Treatment and Diagnostic Center, Ukraine; 12Comprehensive Cancer Centers of Nevada, Henderson, NV, USA; 13Kyiv City Clinical Oncology Center, Ukraine; 14Pfizer Oncology, San Diego, CA, USA

Presented at AACR 2014; April 6, 2014; San Diego, CA USA
Progression-Free Survival (ITT)

<table>
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<tr>
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<th><strong>PAL + LET</strong> (N=84)</th>
<th><strong>LET</strong> (N=81)</th>
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<tr>
<td>Number of Events (%)</td>
<td>41 (49)</td>
<td>59 (73)</td>
</tr>
<tr>
<td>Median PFS, months</td>
<td>20.2 (13.8, 27.5)</td>
<td>10.2 (5.7, 12.6)</td>
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<tr>
<td>Hazard Ratio (95% CI)</td>
<td>0.488 (0.319, 0.748)</td>
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<tr>
<td>p-value</td>
<td>0.0004</td>
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Palbociclib efficacy in ER positive HER2 negative breast cancer

Finn et al AACR 2014
Prognostication is first line metastatic breast cancer

Who will do well on endocrine therapy alone?
Prognostication is first line metastatic breast cancer

Who will do well on endocrine therapy alone?

<table>
<thead>
<tr>
<th>Low risk</th>
<th>High risk</th>
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<tbody>
<tr>
<td>Endocrine naive</td>
<td>Relapse on prior endocrine</td>
</tr>
<tr>
<td>Low CTC/ctDNA</td>
<td>High CTC/ctDNA</td>
</tr>
<tr>
<td>Luminal A biology</td>
<td>Luminal B biology</td>
</tr>
<tr>
<td>Undetectable <em>ESR1</em> mutation</td>
<td>Detectable <em>ESR1</em> mutation</td>
</tr>
</tbody>
</table>

Studies required to demonstrate clinical utility
Prediction of response
Does failure to suppress CTCs indicate resistance?

Smerage et al JCO 2014
Does failure to suppress CTCs indicate resistance?

Failure to suppress CTCs poor prognosis

A

Why is the study negative
- inability to identify those resistant to chemo

Smerage et al JCO 2014
Does failure to suppress CTCs indicate resistance?

Failure to suppress CTCs poor prognosis

Changing chemotherapy makes no difference

Why is the study negative

- inability to identify those resistant to chemo?
- cross-resistance to changed therapy?
Does ctDNA provide the dynamic range to improve on this?
Molecular analysis in metastatic breast cancer

• Repeat *HER2* (and ER) testing of metachronous metastatic disease has become a standard

• Genetic analysis via biopsy or ctDNA for research now
  - Will become standard in the future

• Molecular analysis for prognostication or prediction
  - Promising research tools
  - Clinical utility unproven