Resolving Morphological Intratumour Breast Cancer Heterogeneity by Molecular Approaches: Clinical Implications

José Palacios
Madrid, Spain
Disclosure Information

I hereby declare that I have had business or personal interests in the following industrial enterprises since 1 September 2017:

<table>
<thead>
<tr>
<th>Name of the enterprise / Nature of the interest</th>
<th>None</th>
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</thead>
</table>
INTRATUMOR HETEROGENEITY IN BREAST CANCER

• INTRATUMORAL HETEROGENEITY IN BREAST CANCER PROGRESSION.

• INTRATUMOR HETEROGENEITY OF BIOMARKERS EXPRESSION.

• CLONAL RELATEDNESS OF MULTIPLE BREAST CANCERS (BILATERAL, MULTIFOCAL, RELAPSE).

• CLONAL ANALYSIS OF MIXED DUCTAL-LOBULAR CARCINOMAS.
• The coexistence of subpopulations of cancer cells that differ in their genetic, phenotypic or behavioral characteristics within a given primary tumor, and between a given primary tumor and its metastasis.

• Spatial heterogeneity refers to the genetic variation across different regions within a single tumor.

• Temporal heterogeneity refers to the genetic variation during the course of disease progression.
INTRATUMOR HETEROGENEITY

VARIANT ALLELE FREQUENCY

ITH PATTERNS DIFFER AMONG CANCER TYPES

Is a single tumor biopsy sufficient to portray the mutational landscape of a tumor?

Renal Cancer

Lung Cancer

Gerlinger et al; Nature 2014

Zhang et al; Science 2014
**BIOLOGICAL BASIS OF INTRATUMOR HETEROGENEITY**

**GENOMIC INSTABILITY**

Endogenous and Exogenous Mutational Processes

"Mutational Signature": reflects the imprint of the type of DNA damage that has occurred.

**Single Nucleotide Level**

- **Age-related mutagenesis** (C > T transitions at CpG sites).
- **APOBEC-mediated mutagenesis** (C > T and C > G mutations at TpC sites).
- **Tobacco** (C > A transversions)
- **Drugs** (platinum, temozolamide, etc.).
- **MMRD.**

**Copy Number Level**

- **HRD** (BRCAness, etc.) (allelic imbalance).
- **Chromothripsis.**
- **Whole-genome doublings.**

---

*McGranahan and Swanton; Cancer Cell 2015*
Pan-cancer analysis of intratumor heterogeneity as a prognostic determinant of survival

<table>
<thead>
<tr>
<th>Survival outcome</th>
<th>Covariates</th>
<th>HR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall survival</td>
<td>High ITH</td>
<td>2.50</td>
<td>1.12-5.20</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Stage</td>
<td>-</td>
<td>-</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Receptor status*</td>
<td>-</td>
<td>-</td>
<td>0.002</td>
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</tbody>
</table>

*ER+/Her2-, ER+/Her2+, ER-/Her2+, Triple Negative

Morris et al; Oncotarget 2016
In DCIS-IDC breast cancer, genomic evolution occurred prior to invasion. Invasion involved the co-migration of multiple clones into the adjacent tissues.

Casasent et al; Cell 2018
Lobular carcinomas in situ display intra-lesion genetic heterogeneity and clonal evolution in the progression to invasive lobular carcinoma.
Clonal Evolution in Breast Cancer Revealed by Single Nucleus Genome Sequencing

Mut. Rate = Normal cells (0.6/cell div./exome)

Mut. Rate = 13 X Normal cell (8/cell div./exome)

Wang et al; Nature 2014
Comparative genomic analysis of primary tumors and metastases in breast cancer

Table 2: Concordance between primary tumors and paired metastases for all detected variants

<table>
<thead>
<tr>
<th>Types of mutations</th>
<th>All mutations (N)</th>
<th>Unshared mutations (N)</th>
<th>Shared mutations (N)</th>
<th>Concordance rate</th>
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<tr>
<td>All mutations</td>
<td>499</td>
<td>125</td>
<td>374</td>
<td>75%</td>
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<tr>
<td>Recurrent mutations</td>
<td>39</td>
<td>3</td>
<td>36</td>
<td>92%</td>
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<tr>
<td>Passenger mutations</td>
<td>460</td>
<td>122</td>
<td>338</td>
<td>73%</td>
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</tbody>
</table>

Genes with recurrent amplifications in breast cancer showed 100% (ERBB2, FGFR1), 96% (CCND1), and 88% (MYC) concordance for the amplified/non-amplified status in primary tumors and metastases.

Bertucci et al; Oncotarget 2016
Genetic Heterogeneity in Therapy-Naïve Synchronous Primary Breast Cancers and Their Metastases

Case 7

- **TP53**: TP53 mut, 8p12-11.21, 8q23.3, -24.22, 17p11.2, 17q12 amp
- **WDFY3**:
- **ASB15**: SMAD4 mut, 8q24.22, -24.3 amp, 17p13.1 and 20p13 hom del
- **WDR5**: P
- **SLC5A11**: P
- **SMAD4**: M
- **PSIP1**: M

**JAK3** (Case 4, E1033D+LOH)
**TCF7L2** (Case 5, S122*)
**SMAD4** (Case 7, D355G+LOH)
**TCF4** (Case 9, c.379-1G>C+LOH)

\[ p < 0.0001 \]

- Likely pathogenic mutations enriched in metastases (n=22)
- Genes associated with EMT (n=187)

Ng et al; Clin Cancer Res 2017
INTRATUMOR HETEROGENEITY IN BREAST CANCER

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• INTRATUMOR HETEROGENEITY OF BIOMARKERS EXPRESSION

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• CLONAL ANALYSIS OF MIXED DUCTAL-LOBULAR CARCINOMAS
Estradiol receptors. HGUA n=1161 cases

% cells | % cases
---|---
0 | 21.6
1-10 | 4.0
11-20 | 3.7
21-30 | 2.3
31-40 | 3.5
41-50 | 3.7
51-60 | 6.4
61-70 | 8.5
71-80 | 10.9
81-90 | 16.6
91-100 | 18.6

Mean = 57,2179
Std. Dev. = 38,3486
N = 1.161

Aranda. Breast Pathology Course. Madrid 2014
Intratumor Heterogeneity of the Estrogen Receptor and the Long-term Risk of Fatal Breast Cancer

HER2 HETEROGENEITY
HER2 intratumoral heterogeneity is independently associated with incomplete response to anti-HER2 neoadjuvant chemotherapy in HER2-positive breast carcinoma.

Hou Y¹, Nitta H², Wei L³, Banks PM², Portier B², Parwani AV¹, Li Z⁴.

Abstract

PURPOSE: Anti-HER2 neoadjuvant chemotherapy has been widely used in HER2-positive breast cancer patients; however, pathologic complete response (pCR) is achieved in only 40-50% of patients. The aim of this study was to investigate the association of HER2 intratumoral heterogeneity (ITH) with response to anti-HER2 neoadjuvant chemotherapy.

METHODS: Assessment of HER2 ITH was performed on whole tissue sections of pre-treatment samples from a cohort of 64 invasive breast carcinoma cases originally considered positive for HER2 and treated with anti-HER2 neoadjuvant chemotherapy. Both HER2 gene signal and protein expression were simultaneously evaluated by means of a single-slide dual assay, designated as a HER2 gene-protein assay (GPA). HER2 GPA was carried out as well on surgical resection tissues from 25 cases with incomplete therapeutic response.

RESULTS: Nineteen of 64 cases (30%) showed HER2 ITH. Significantly more cases with HER2 ITH were found in the incomplete response group (56%, 14/25) than in the pCR group (13%, 5/39). Patients without ITH detectable by GPA had a 76% pCR outcome (34/45), as compared to 26% (5/19) for those with detectable ITH. Multivariate analysis demonstrated HER2 ITH, progesterone receptor positivity, and relatively low HER2/chromosome 17 centromere ratio to be significantly associated with incomplete response.

CONCLUSIONS: HER2 ITH analyses conducted with GPA method revealed that HER2 ITH is an independent factor predicting incomplete response to anti-HER2 neoadjuvant chemotherapy.
HETEROGENEITY IN BIOMARKER EXPRESSION AMONG MULTIFOCAL BREAST TUMORS

20-25% of BC are multifocal
Multifocality is not associated with specific molecular subtypes

<table>
<thead>
<tr>
<th>Author</th>
<th>Sample</th>
<th>ER</th>
<th>PR</th>
<th>HER2</th>
<th>Phenotype</th>
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<tbody>
<tr>
<td>Buggi et al, 2012</td>
<td>113</td>
<td>4%</td>
<td>15%</td>
<td>9%</td>
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<tr>
<td>Choi et al., 2012</td>
<td>65</td>
<td>3%</td>
<td>11%</td>
<td>6%</td>
<td>8%</td>
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<tr>
<td>Pekar et al; 2012</td>
<td>110</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10%</td>
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# PROSPECTIVE CONVERSION STUDIES IN METASTATIC BREAST CANCER

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<tr>
<th>Study</th>
<th>Sample</th>
<th>ER</th>
<th>PR</th>
<th>HER2</th>
<th>Change treatment in discordant cases</th>
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<tbody>
<tr>
<td>BRITS</td>
<td>137</td>
<td>10%</td>
<td>25%</td>
<td>3%</td>
<td>49%</td>
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<tr>
<td>Thompson et al., 2010</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>DESTINY</td>
<td>121</td>
<td>16%</td>
<td>40%</td>
<td>10%</td>
<td>39%</td>
</tr>
<tr>
<td>Amir et al, 2012</td>
<td></td>
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<tr>
<td>CONVERTHER</td>
<td>184</td>
<td>13%</td>
<td>28%</td>
<td>3%</td>
<td>31%</td>
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<tr>
<td>Mátinez de Dueñas et al; 2014</td>
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</tbody>
</table>
BIOMARKERS CONVERSION AFTER NAC

- RE change (13-18%)
- RP change (26-32%)
- Her-2 change (6-9%)
  - Her-2 lost associates to poor prognosis
- High Ki67 associates to poor prognosis.
- Change to TNBC (10%) associates to poor prognosis.
- TILs.
- There is not a formal recommendation to analyse predictive markers after neoadjuvant therapy.

Von Minckwitz G et al 2012
Jin et al 2015
Provenzano E, et al, 2015
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A cancer in the contralateral breast in a woman with a previous or synchronous breast cancer is typically considered to be an independent primary tumor.

Emerging evidence suggests that in a small subset of these cases the second tumor represents a metastasis.

6%-29% of bilateral cancers are clonally related.
MULTIFOCAL BREAST CANCER: SINGLE OR MULTIPLE PRIMARIES?

“Our Ipsiliteral Synchronous cohort showed clonality in 64% (7/11) of the patients”

Biermann et al; Breast Cancer Res 2018
Uncovering the genomic heterogeneity of multifocal breast cancer
Desmedt et al; J Pathol 2015

36 Multifocal IDC with the same grade, ER and HER2 profile

31% homeogenous
36% intermediate
33% heterogeneous

Clonally related
Clonally unrelated
• Some patients who present with an IBTR after conservative surgery with or without RT may have a new primary tumor as opposed to a true local recurrence.

• The diagnosis of a new primary as opposed to a true recurrence implies a different natural history and prognosis and has implications for therapeutic management.
TRUE RECURRENCE VERSUS NEW PRIMARY: AN ANALYSIS OF IPSILATERAL BREAST TUMOR RECURRENCES AFTER BREAST-CONSERVING THERAPY

2005

2016

RE

p53
<table>
<thead>
<tr>
<th>Year</th>
<th>KMT2C</th>
<th>KRAS</th>
<th>MAP3K1</th>
<th>NCOR</th>
<th>PIK3CA</th>
<th>TP53</th>
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<td>05-T1</td>
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<td>del442G; loss</td>
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<td>05-T2</td>
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<td>del442-458 (17pb)</td>
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<td>16-T1</td>
<td>E1253*</td>
<td></td>
<td>K391*; del1144-1145 (6pb)</td>
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<td></td>
<td>E545K</td>
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<td>2016</td>
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<tr>
<td></td>
<td>IDC (97)</td>
<td>IDC (2003)</td>
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<tr>
<td><em><em>BRCA2-K3326</em>(Hetero)</em>*</td>
<td><em><em>BRCA2-K3326</em>(Hetero)</em>*</td>
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<td>loss6q (ESR1-ARID1B)</td>
<td>loss6q (ESR1-ARID1B)</td>
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<td>loss12q (TB233)</td>
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<td>amp17q (ERBB2-GRB7)</td>
<td>amp11q (CCND1)</td>
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<td>loss12p (KRAS)</td>
<td>gain10p (GATA3)</td>
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<td>loss1p (ARID1A)</td>
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<td>loss16q (CDH1)</td>
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<tr>
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<td>lossXq (VGLL1)</td>
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</tbody>
</table>

### Diagrams

- **Top Diagram:**
  - Highlighted gene: **ERBB2**
  - Axes: X-axis, Y-axis

- **Bottom Diagram:**
  - Highlighted gene: **ERBB2**
  - Axes: X-axis, Y-axis
• Intratumoral heterogeneity in breast cancer and tumor progression.

• Intratumor heterogeneity of biomarkers expression.

• Clonal relatedness of multiple breast cancers (bilateral, multifocal, relapse).

• Clonal analysis of mixed ductal-lobular carcinomas.
MIXED LOBULAR/DUCTAL CARCINOMAS

<5% of breast carcinomas have histologic features of both ductal and lobular invasive ductal carcinomas

Collision tumors?  
Clonally related?
<table>
<thead>
<tr>
<th>Case</th>
<th>CDH1</th>
<th>AKT1</th>
<th>GATA3</th>
<th>PIK3CA</th>
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<td>T1-D</td>
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<tr>
<td>T1-L</td>
<td>c.1351delA</td>
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<td>---------------</td>
<td>---------</td>
</tr>
<tr>
<td>T2-D</td>
<td>N2830H / R2013K</td>
<td>Y1021C</td>
<td>C176F</td>
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<td>c.2029delC</td>
<td>N2830H / R2013K</td>
<td>C176F</td>
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</table>
# NGS ANALYSIS OF MIXED LOBULAR/DUCTAL CARCINOMAS

<table>
<thead>
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<th>Case</th>
<th>CDH1</th>
<th>AKT1</th>
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Mixed ductal–lobular carcinomas: evidence for progression from ductal to lobular morphology

Reed et al; J Pathol 2018
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