Disclosure Information

I hereby declare that I have had business or personal interests in the following industrial enterprises since 1 September 2016:

Name of the enterprise / Nature of the interest

<table>
<thead>
<tr>
<th>Enterprise</th>
<th>Interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Employment: Scientific Fellow, Ventana Medical Systems, Inc., A Member of Roche Group</td>
<td></td>
</tr>
</tbody>
</table>
HER2 status evaluation of residual breast cancer after neoadjuvant trastuzumab-based chemotherapy with a combined gene-protein assay

Hiroaki Nitta, Bryce Portier, Peter M Banks, Zaibo Li
Efficacy of trastuzumab-based neoadjuvant chemotherapy for HER2 positive breast cancer

Neoadjuvant combination of trastuzumab and chemotherapy results in a high rate of pathological complete response (pCR) rate in HER2 positive breast cancer

- Patients with pCR have a favorable long-term outcome
- Patients without pCR have an increased risk for relapse and death

Current definition of breast HER2 intratumoral heterogeneity for ASCO-CAP guidelines

HER2 genetic heterogeneity exists if there are more than 5% but less than 50% of tumor cells with a $HER2/CEN17$ ratio higher than 2.2

$CEN17$ chromosome 17 centromere

Vance GH et al., *Arch Pathol Lab* (2009) 133, 611-612

Hanna WM et al., *Mod Pathol* (2014) 27, 4-18
HER2 gene-protein assay (GPA)

Detection 1:
- HER2
- Immunohistochemistry
- Primary Antibody Incubation → Secondary Antibody Incubation → HRP Labeling → DAB Detection

Detection 2:
- HER2
- Brightfield
- In Situ Hybridization
- Probe Co-Hybridization with Naphthol Phosphate → Primary Antibody Incubation → Secondary Antibody Incubation → Silver Detection

Detection 3:
- CEN17
- Brightfield
- In Situ Hybridization
- Primary Antibody Incubation → Secondary Antibody Incubation → Red Detection

Nitta et al., Diagn Pathol (2012) 30;7:60
Concurrent demonstration of HER2 gene, CEN17, and HER2 protein

A. HER2 gene +, HER2 protein +
B. HER2 gene +, HER2 protein -
C. HER2 gene +, HER2 protein -

Homogeneity | Genetic heterogeneity | Epigenetic heterogeneity
---|---|---
HER2 gene + | HER2 gene + | HER2 gene +
HER2 protein + | HER2 protein - | HER2 protein -
X | X | X

- A: HER2 gene +, HER2 protein +
- B: HER2 gene +, HER2 protein -
- C: HER2 gene +, HER2 protein -
Our definition of HER2 epigenetic heterogeneity

HER2 epigenetic heterogeneity indicates the presence of \textit{HER2} gene amplified tumor cells without HER2 protein overexpression.
Materials and Methods

Patient cases
64 HER2 positive invasive breast cancer patients
- anti-HER2 neoadjuvant chemotherapy
  57 patients; 4 cycles of doxorubicin+cyclophosphamide+paclitaxel+docetaxel+trastuzumab
  7 patients; 4 cycles of doxorubicin+cyclophosphamide+docetaxel+trastuzumab+pertuzumab
  ● follow-up surgical resection

HER2 GPA
HER2 gene and HER2 protein status
HER2 heterogeneity status

pCR definition
No detectable residual invasive tumor with exhaustive sampling and absence of lymph nodal metastasis

Statistical analyses
Multivariate analyses
Multivariate analyses of factors associated with incomplete response to anti-HER2 targeted therapy

<table>
<thead>
<tr>
<th></th>
<th>pCR</th>
<th>Non-pCR</th>
<th>p value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#/median</td>
<td>%/range</td>
<td>#/median</td>
<td>%/range</td>
</tr>
<tr>
<td>Case #</td>
<td>39</td>
<td></td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>ER-positive</td>
<td>14</td>
<td>36%</td>
<td>17</td>
<td>68%</td>
</tr>
<tr>
<td>PR-positive</td>
<td>7</td>
<td>18%</td>
<td>13</td>
<td>52%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2 signals/cell</td>
<td>20.1</td>
<td>3.6-35.4</td>
<td>12.8</td>
<td>3.2-40.3</td>
</tr>
<tr>
<td>HER2/CEN17 ratio</td>
<td>7.6</td>
<td>2.3-23.0</td>
<td>4.6</td>
<td>1.2-15.0</td>
</tr>
<tr>
<td>HER2 heterogeneity</td>
<td>5</td>
<td>13%</td>
<td>14</td>
<td>56%</td>
</tr>
</tbody>
</table>

*pCR* pathological complete response, *OR* odds ratio, *ER* estrogen receptor, *PR* progesterone receptor, *CEN17* chromosome 17 centromere
Distribution of HER2 homogeneity and heterogeneity comparing pCR and non-pCR pre-treatment specimens

Distribution of HER2 homogeneity and heterogeneity comparing pCR and non-pCR pre-treatment specimens

- **pCR**: 61% (39/64)
  - Genetic & epigenetic heterogeneity: 11%
  - Epigenetic heterogeneity: 2%
  - Genetic heterogeneity: 1%
  - Homogeneity: 57%

- **LRTB**: 28% (18/64)
  - Genetic & epigenetic heterogeneity: 11%
  - Epigenetic heterogeneity: 3%
  - Genetic heterogeneity: 2%
  - Homogeneity: 84%

- **HRTB**: 11% (7/64)
  - Genetic & epigenetic heterogeneity: 10%
  - Epigenetic heterogeneity: 2%
  - Genetic heterogeneity: 1%
  - Homogeneity: 87%

LRTB: low residual tumor burden, HRTB: high residual tumor burden
Distribution of HER2 homogeneity and heterogeneity comparing pCR and non-pCR pre-treatment specimens

<table>
<thead>
<tr>
<th>Case number (n)</th>
<th>Heterogeneity</th>
<th>Non-pCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCR</td>
<td>15% (5/39)</td>
<td>61% (39/64)</td>
</tr>
<tr>
<td>LRTB</td>
<td>50% (9/18)</td>
<td>28% (18/64)</td>
</tr>
<tr>
<td>HRTB</td>
<td>71% (5/7)</td>
<td>11% (7/64)</td>
</tr>
</tbody>
</table>

LRTB low residual tumor burden, HRTB high residual tumor burden
Distribution of HER2 homogeneity and heterogeneity comparing pCR and non-pCR pre-treatment specimens

<table>
<thead>
<tr>
<th></th>
<th>pCR</th>
<th>LRTB</th>
<th>HRTB</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2 protein loss</td>
<td>33% (5/18)</td>
<td>50% (9/18)</td>
<td>14% (1/7)</td>
</tr>
<tr>
<td>Genetic &amp; epigenetic heterogeneity</td>
<td>15% (5/39)</td>
<td>71% (5/7)</td>
<td></td>
</tr>
<tr>
<td>Epigenetic heterogeneity</td>
<td></td>
<td></td>
<td>11% (1/7)</td>
</tr>
<tr>
<td>Genetic heterogeneity</td>
<td></td>
<td></td>
<td>50% (9/18)</td>
</tr>
<tr>
<td>Homogeneity</td>
<td>61% (39/64)</td>
<td>28% (18/64)</td>
<td>11% (7/64)</td>
</tr>
</tbody>
</table>

LRTB low residual tumor burden, HRTB high residual tumor burden
HER2 GPA analyses: pCR patients’ pretreatment specimens

Homogeneity
(Case# 108)

Genetic heterogeneity
(Case# 98)

Objective 10X
Objective 60X

HER2 gene & HER2 protein
positive tumor cells

HER2 negative
tumor cells

Homogeneity
(Case# 108)

Genetic heterogeneity
(Case# 98)
Objective 60X
HER2 GPA analyses: non-pCR patients

Low residual tumor burden - Pre-treatment

Epigenetic heterogeneity
(Case# 100)
HER2 GPA analyses: non-pCR patients

Low residual tumor burden - Post-treatment

Epigenetic heterogeneity

(Case# 100)
Trastuzumab-based neoadjuvant chemotherapy efficacy model

HER2 homogeneity
Pre-treatment
Chemotherapy sensitive
Post-treatment

HER2 genetic heterogeneity
Trastuzumab sensitive

HER2 epigenetic heterogeneity
Trastuzumab resistant

Longer therapy for eradicating tumor cells?
Conclusions

Neoadjuvant HER2-targeted therapy effected reduction/elimination of HER2 protein-overexpressed tumor cells even among non-pCR patients.

Some patients may need longer HER2 targeted-therapy for achieving pCR.

It would appear, however, that loss of HER2 protein expression is a mechanism for breast cancer resistance to trastuzumab-based therapy.