Breast cancer metastases: A spitting image of their primary?
Breast cancer is the leading cause of female cancer death worldwide (13,000 new cases yearly in The Netherlands).

One third of patients will develop distant metastases (bone, liver, lungs, skin, brain).

Distant metastases necessitate systemic therapy:
- Chemotherapy
- Hormonal therapy targeting
  - estrogen receptor (ERα)
  - progesterone receptor (PR)
- Antibody therapies targeting e.g. human epidermal growth factor receptor 2 (HER2)
Systemic therapy for metastases

- Therapy choice now personalized on the basis of immunophenotype of primary tumor
- Metastases generally not biopsied (inconvenient/inaccessible/deemed unnecessary)

Previous studies* indicate that immunophenotype of breast cancer metastases may differ from the primary tumor (“receptor conversion”)

- ER % conversion rates: 12-57%
- PR % conversion rates: 28.3-61%
- HER2 % conversion rates: 0-44%

This means that many metastatic patients may not get the appropriate systemic treatment when based on the primary tumor!

  Tanner M et al. Cancer Res 2001
  Broom RJ et al. Anticancer res 2009
Aim

So:

• Studies so far are small
• Just one metastatic site
• Ligand-binding assay used
• TMA
• Bone metastases included (false negativity?)
• Original primary tumor staining used!

Aim:

To study frequency of receptor conversion for ER, PR, and HER2:
  – Large group of breast cancer metastases
  – Different sites (brain, liver, lung, skin, GI)
  – No bone metastases
  – New stainings, one protocol
  – Full sections
Material and Methods

• 236 paired samples of primary breast cancer and corresponding distant metastases ("Dutch breast cancer metastases consortium")

• Sites: 44 brain, 43 lung, 63 liver, 79 skin, 7 gastro-intestinal

• Immunohistochemistry (IHC) for ERα, PR and HER2

• HER2 silver in situ hybridization (SISH) was done in cases of IHC conversion or when primary tumors or metastases were IHC 2+

• Whole sections, fresh immunohistochemical staining
Receptor conversion in distant breast cancer metastases

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Results

ER

primary

metastasis

liver

brain

skin

liver

PR

HER2
### Results: conversion for ER and PR

10% threshold

<table>
<thead>
<tr>
<th>Primary tumor</th>
<th>Metastasis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ERα</td>
<td>79 (33.9%)</td>
<td>7 (3.0%)</td>
</tr>
<tr>
<td></td>
<td>17 (7.2%)</td>
<td>130 (55.8%)</td>
</tr>
<tr>
<td>PR</td>
<td>92 (39.5%)</td>
<td>12 (5.1%)</td>
</tr>
<tr>
<td></td>
<td>58 (24.9%)</td>
<td>71 (30.5%)</td>
</tr>
</tbody>
</table>

In total: conversion rate for ER 10.2%, for PR 30%
### Results: conversion for ER and PR

#### 1% threshold

<table>
<thead>
<tr>
<th>Primary tumor</th>
<th>Metastasis</th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERα</td>
<td>47 (20.2%)</td>
<td>12 (5.2%)</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23 (9.9%)</td>
<td>151 (64.8%)</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>33 (14.2%)</td>
<td>27 (11.6%)</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49 (21.0%)</td>
<td>124 (52.2%)</td>
<td>173</td>
<td></td>
</tr>
</tbody>
</table>

In total: conversion rate for ER 15.1%, for PR 32.6%
Results: HER2

ERα, PR and HER2 expression by IHC in paired primary tumors and distant breast cancer metastases

<table>
<thead>
<tr>
<th>Primary tumor</th>
<th>Metastasis</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>180 (77.2%)</td>
<td>6 (2.6%)</td>
</tr>
<tr>
<td>HER2 IHC</td>
<td>-</td>
<td>180 (77.2%)</td>
<td>6 (2.6%)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>6 (2.6%)</td>
<td>41 (17.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>186</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>47</td>
</tr>
</tbody>
</table>

Of the 12 cases with HER2 conversion by IHC, 5 also showed conversion by SISH. One further case showed conversion by SISH but not by IHC.
## Results: conversion rates per metastatic site

<table>
<thead>
<tr>
<th></th>
<th>% conversion</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ERα</td>
<td>PR</td>
<td>HER2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>N (%)</td>
<td>95% CI</td>
<td>N (%)</td>
</tr>
<tr>
<td>Brain</td>
<td>44</td>
<td>6 (13.7)</td>
<td>5.2-27.3</td>
<td>16 (36.3)</td>
</tr>
<tr>
<td>Lung</td>
<td>43</td>
<td>4 (9.4)</td>
<td>2.6-22.1</td>
<td>8 (18.6)</td>
</tr>
<tr>
<td>Liver</td>
<td>63</td>
<td>8 (12.7)</td>
<td>5.7-23.5</td>
<td>26 (41.2)</td>
</tr>
<tr>
<td>Skin</td>
<td>79</td>
<td>5 (6.3)</td>
<td>2.1-14.2</td>
<td>17 (21.5)</td>
</tr>
<tr>
<td>Gastro-intestinal</td>
<td>7</td>
<td>1 (14.3)</td>
<td>0.36-57.9</td>
<td>3 (42.9)</td>
</tr>
</tbody>
</table>

→ Receptor conversion seemed to occur mostly in liver and brain metastases for ER and PR, and in liver metastases for HER2 (GI?)
Clinically, tamoxifen is given when primary tumor is either ER+ or PR+, and withheld when ER and PR are both negative.

So, when basing tamoxifen therapy on immunophenotype of primary tumor, clinically there are 2 problematic situations:

- ER or PR+ → ER-/PR- OR ER-/PR- → ER+ or PR+

10% threshold:
- ER- primary and ER+ or PR+ metastases: 3.4%
- ER+ or PR+ primary and ER-/PR- metastases: 10.7%

14%

1% threshold:
- ER-/PR- primary and ER+ or PR+ metastases: 8.2%
- ER+ or PR+ primary and ER-/PR- metastases: 12.4%

21%
Results: Heterogeneity between metastases

40 cases with multiple metastases, 26 analyzed

<table>
<thead>
<tr>
<th>Receptor</th>
<th>homogeneous</th>
<th>heterogeneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>PR</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td>HER2</td>
<td>26</td>
<td>0</td>
</tr>
</tbody>
</table>
### Results: receptor conversion due to tamoxifen treatment?

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Threshold</th>
<th>Hormonal therapy</th>
<th>% conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>ER</td>
<td>10%</td>
<td>no</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>yes</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>no</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>yes</td>
<td>95</td>
</tr>
<tr>
<td>PR</td>
<td>10%</td>
<td>no</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>yes</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>no</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>yes</td>
<td>80</td>
</tr>
</tbody>
</table>
Results: Prognostic value of ER conversion

1% threshold

Cumulative survival over survival time (days)

- Conversion - --> +
- Conversion + --> -
- No conversion

p < 0.001
Results: Prognostic value of PR conversion

1% threshold

Survival time (days)

Cum survival

Conversion + --> -

Conversion - --> +

No conversion

p=0.011
Results: Prognostic value of ER/PR conversion

1% threshold

p<0.001
Results: Prognostic value of HER2 conversion

Comparison of survival curves for HER2 conversion status:
- IHC: $p = 0.253$
- ISH: $p = 0.374$
Conclusions: not a spitting image!

- Frequency of receptor conversion in distant breast cancer metastases: ERα 10-15%, PR 30-33%, HER2 3-5%
- Receptor conversion is seen especially in brain and liver metastases
- In approximately 16-26% of patients such conversion would have direct consequences for the therapeutic regimen
- Adjuvant tamoxifen treatment does not seem to induce receptor conversion
- Heterogeneity between metastases from the same patient seems limited
Conclusions

• Receptor conversion has negative prognostic impact

• These results underline the importance of having distant breast cancer metastases biopsied when possible

• Alternatively, molecular imaging strategies should be explored for sites that are difficult to biopsy

PET imaging with $^{89}$Zr-Trastuzumab    PET imaging with $^{18}$Fluoroestradiol

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