INCREASED LONG-TERM RISK OF FATAL BREAST CANCER IN PATIENTS WITH HIGH INTRA-TUMOR HETEROGENEITY OF THE ESTROGEN RECEPTOR

– Retrospective analyses of the STO-3 trial

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DISCLOSURE SLIDE

No conflicts of interest to declare.
BACKGROUND

• We and others have shown that the estrogen receptor (ER) expression in primary breast cancer tumors is altered throughout tumor progression, which significantly influences patient survival (Lindström et al, JCO 2012)

PURPOSE

• To determine whether high intra-tumor heterogeneity of the ER is associated with an increased long-term risk (25 years) of fatal breast cancer in patients with ER-positive disease
PATIENTS AND METHODS

The Stockholm tamoxifen (STO-3) trial
• Enrolled breast cancer patients between 1976 until the end of 1990
  - Postmenopausal, lymph node-negative, and tumor size of less than 30 mm
  - Randomized to receive adjuvant tamoxifen versus not (all patients were operated)

Intra-tumor heterogeneity of ER
• Whole tumor ER stained slides (re-stained in 2014) were scored by breast cancer pathologists assessing the percentage of cancer cells for each ER intensity level
• Intra-tumor heterogeneity was defined as differences in ER intensity levels (0,+1,+2,+3) within a whole tumor section creating a score called Rao’s quadratic entropy
• Rao’s quadratic entropy (QE)
  - Is defined as Simpson index (Simpson EH, Nature 1949) with the addition of a distance matrix
• The predefined cut-off at the third tertile for high intra-tumor heterogeneity was used
STATISTICAL METHODS

• All patients have detailed patient and clinical information along with a complete 25 year long-term follow-up (National Swedish registers with high validity and coverage)

• Analysis were performed for:
  - Patients with ER-positive disease
  - Patients with Luminal A subtype tumors (Agilent gene expression arrays)

Kaplan Meier analyses
• High versus low intra-tumor heterogeneity of ER by STO-3 trial arm

Multivariable Cox proportional hazard analyses
• High versus low (reference) intra-tumor heterogeneity of ER adjusting for classical patient and tumor characteristics
  - Age and year of breast cancer diagnosis, ER-positive stained cells (%), ER H-Score, PR status, HER2 status, Ki-67 status, tumor grade, tumor size and STO-3 trial arm
RESULTS

ER immunohistochemistry in four representative patients

A: Low intra-tumor heterogeneity of ER and high ER H-score

B: High intra-tumor heterogeneity of ER and high ER H-score

C: Low intra-tumor heterogeneity of ER and low ER H-score

D: High intra-tumor heterogeneity of ER and low ER H-score
RESULTS

Patients with ER-positive tumors

A statistically significant difference in long-term survival with intra-tumor heterogeneity of ER and trial arm was seen (Log rank, P<0.0001)

Risk (Hazard ratio) of long-term breast cancer-specific death (25 years) by intra-tumor heterogeneity of ER*

<table>
<thead>
<tr>
<th></th>
<th>Hazard Ratio (95% CI)</th>
<th>Low ER het - Treated</th>
<th>Low ER het - Untreated arm</th>
<th>High ER het - Treated arm</th>
<th>High ER het - Untreated arm</th>
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<tbody>
<tr>
<td>High</td>
<td>1.98 (1.31-3.00)</td>
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<tr>
<td>Low</td>
<td>1.0 ref.</td>
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*Adjusting for patient and tumor characteristics

Numbers at risk

- Low ER heterogeneity/Treated: 205, 189, 165, 130, 95, 48
- High ER heterogeneity/Treated: 102, 95, 83, 70, 52, 22
- High ER heterogeneity/Untreated: 94, 82, 62, 47, 32, 14
RESULTS

Patients with Luminal A tumors

A statistically significant difference in long-term survival with intra-tumor heterogeneity of ER and trial arm was seen (Log rank, P=0.011)

Risk of long-term breast cancer-specific death (25 years) by intra-tumor heterogeneity of ER*

- **High**: 2.43 (1.18-4.99)
- **Low**: 1.0 ref.

*Adjusting for patient and tumor characteristics

**Numbers at risk**

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<thead>
<tr>
<th></th>
<th>Treated</th>
<th>Untreated</th>
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<tr>
<td>Low ER heterogeneity</td>
<td>126</td>
<td>107</td>
</tr>
<tr>
<td>Low ER untreated</td>
<td>111</td>
<td>98</td>
</tr>
<tr>
<td>High ER heterogeneity</td>
<td>57</td>
<td>46</td>
</tr>
<tr>
<td>High ER untreated</td>
<td>100</td>
<td>33</td>
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![Graph showing survival rates with log rank P=0.011]
CONCLUSIONS

- Patients with high intra-tumor heterogeneity of ER had an increased long-term risk of fatal breast cancer as compared to patients with low intra-tumor heterogeneity.

- Interestingly, a similar long-term risk increase was seen in patients with Luminal A subtype tumors.

- Therefore, routine clinical assessment of intra-tumor heterogeneity of ER may identify patients at high long-term risk for fatal breast cancer, which may potentially change clinical management especially for patients with Luminal A subtype tumors.
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