Molecular predictors of outcome on adjuvant CAF plus tamoxifen versus tamoxifen alone in postmenopausal patients with ER+, node+ breast cancer: Transcriptome expression analysis of the phase III trial SWOG S814

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BACKGROUND

- SWOG S8814 (INT0100) showed that postmenopausal patients with ER+, node+ tumors had superior disease-free survival (DFS) from CAF chemotherapy\(^1\) followed by tamoxifen for 5 years versus tamoxifen\(^2\).
- A biomarker study (SWOG S8814A) reported good prognosis and no CAF benefit for patients whose cancers had low 21-gene Recurrence Score (RS) results, compared to significant DFS benefit from CAF if high RS\(^3\).

\(^{1}\)CAF: cyclophosphamide (cmp), Adriamycin, 5-Fluorouracil x 5 cycles.
\(^{3}\)Albain K.S. et al. Lancet Oncology, 2010

OBJECTIVES – New Biomarker Study (SWOG S8814B)

- Conduct RNA sequencing (RNA Seq) with next generation sequencing (NGS) technology
- Utilize whole transcriptome analysis to discover novel genes/networks associated with prognosis and prediction:
  - Prognosis for relapse on the tamoxifen-alone arm – overall and for early (years 0-5) and late (beyond 5 years) time periods
  - Prediction of CAF benefit
- Refine identification of a node-positive cohort that can avoid chemotherapy
METHODS – I

• RNA-Seq applied to RNA stored from prior biomarker study (S8814A)
• Gene expression related to DFS used Cox proportional hazards regression
• False discovery rate (FDR) controlled at 10%
• Discovery of networks of genes and pathways via functional gene and metagene analyses used:
  - Unsupervised hierarchical clustering
  - Principal Components
  - Gene Ontology, Cytoscape and Pathway Analysis
• Details provided in Diana Cherbavaz et al. Friday evening poster session

METHODS - II

Cytoscape illustrates the inter-relationships of the discovered genes to visualize biologic networks

• Gene clusters with same function (a circle)
• Over-represented (larger size of circles)
• Relationship (distance)
• Direction of regulation (arrows)
• Significance (color: white-yellow-orange)

Not significant                Highly significant

p-value:          >5x10⁻² ≤ 5x10⁻² <5x10⁻²

• Metagenes derived from closely related gene groups (within rectangle)
RESULTS –
Gene Discovery and Prognosis

High success rate for sequencing the archived samples from SWOG S8814

- All 367 samples banked from prior biomarker study available
- 354 (96%) had sufficient RNA/library yield
- 142 tamoxifen alone, 212 CAF-tamoxifen (well-balanced for age, nodes, grade, T size)
- Over 20,000 genes (exonic sequences) analyzed for discovery
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Genes prognostic for DFS (better, worse) varied by time period in the tamoxifen-alone arm (n =142)

- 568 genes prognostic for all follow-up
- More genes prognostic for early recurrence, fewer for late events
- 2327 genes prognostic during years 0-5*
- 9 genes prognostic beyond 5 years

<table>
<thead>
<tr>
<th>Follow-up time period</th>
<th>Genes better DFS</th>
<th>Genes worse DFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>All years</td>
<td>265</td>
<td>303</td>
</tr>
<tr>
<td>Early (0-5 years)</td>
<td>1226</td>
<td>1101</td>
</tr>
<tr>
<td>Late (&gt;5 years)</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

*All genes after FDR adjustment

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Genes prognostic for worse DFS (tamoxifen arm) enriched for proliferation, DNA repair, EMT, cellular metabolism and stress response metagenes

Epithelial-mesenchymal transition (EMT)

DNA repair

Proliferation (G2/M, M)

Cellular metabolism

Stress response

(a 23-gene metagenes)
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Metagenes prognostic for better DFS* (tamoxifen arm) involved transcription regulation/repression via zinc finger proteins

*CytoScape, all years follow-up

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Unsupervised hierarchical clustering identified significant prognostic metagene signatures over time for DFS in the tamoxifen-alone arm

Low ESR1 was associated with early recurrence... but high proliferation impacted early and late events
Early versus late DFS events were best defined by ESR1 plus proliferation metagene categories
[rates of recurrence/death (tamoxifen-alone arm) per patient-year, adjusted for nodes]

- Low ESR1 significantly associated only with early recurrence
- High proliferation significantly associated with early and late events

5-metagene Cox multivariate model best defined prognosis for DFS events over 10 years in the tamoxifen-alone arm

<table>
<thead>
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<th>Effect</th>
<th>Hazard Ratio (95% CI)</th>
<th>Wald p-value</th>
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<td>ESR1 (standardized)</td>
<td>0.58 (0.36, 0.92)</td>
<td>0.020</td>
</tr>
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<td>Proliferation</td>
<td>2.20 (1.59, 3.06)</td>
<td>&lt;.001</td>
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<td>ECM/stroma (standardized)</td>
<td>1.15 (0.84, 1.57)</td>
<td>0.38</td>
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<tr>
<td>Immune (standardized)</td>
<td>1.03 (0.82, 1.30)</td>
<td>0.80</td>
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<tr>
<td>TGFβ (standardized)</td>
<td>0.88 (0.68, 1.15)</td>
<td>0.36</td>
</tr>
<tr>
<td>Nodes (4+ vs 1-3)</td>
<td>2.82 (1.71, 4.65)</td>
<td>&lt;.001</td>
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5-metagene Cox multivariate model best defined prognosis for DFS events over 10 years in the tamoxifen-alone arm

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10-yr risk of recurrence/death estimated from this model, divided into low risk (≤15%) vs. high risk (>15%)

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Excellent long term DFS in tamoxifen-alone arm for the low risk subset defined by the 5-metagene model

Low 10-year risk (≤15%) versus high 10-year risk (>15%)

*Only 1 patient in low risk subset had 4+ positive nodes
RESULTS –
Prediction of Benefit from Chemotherapy

Genes were discovered for prediction of chemotherapy benefit only in the first 5-years*, with the number of genes varying by false discovery rate

<table>
<thead>
<tr>
<th>Number of Genes Discovered</th>
<th>FDR 10%</th>
<th>Total</th>
<th>Chemotherapy More Effective</th>
<th>Chemotherapy Less Effective</th>
</tr>
</thead>
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<tr>
<td>10%</td>
<td></td>
<td>15</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>20%</td>
<td>129</td>
<td></td>
<td>73</td>
<td>56</td>
</tr>
</tbody>
</table>

*No genes discovered beyond 5 years
Of the 5 metagenes, three best defined prediction of chemotherapy benefit in the Cox multivariate model
[all patients (n=354), all follow-up]

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<th>Effect (Standardized)</th>
<th>Interaction Hazard Ratio (95% CI)</th>
<th>Wald p-value</th>
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<tr>
<td>Proliferation x tx</td>
<td>0.52 (0.35, 0.75)</td>
<td>0.001</td>
</tr>
<tr>
<td>TGFβ3 x tx</td>
<td>1.63 (1.04, 2.57)</td>
<td>0.024</td>
</tr>
</tbody>
</table>

- If HR>1, higher expression means chemotherapy less effective, and vice versa;
- tx, treatment

- Cox model also had effects for nodal status and ESR1 metagene
- Stromal and immune metagenes were not significantly predictive

Thus, 3 metagenes were optimal for prediction
CONCLUSIONS - I

- Unique genes, clusters and pathways (including previously unreported metagene signatures) were identified by RNA Seq in archival material from an ER+, node+ breast cancer trial (SWOG S8814).
- Analysis of the primary tumor found many single genes prognostic for early DFS events on tamoxifen alone for 5 years, but few single genes were prognostic for late events.
CONCLUSIONS - II

- However, distinct metagenes and pathways were very informative for good versus poor prognosis in the tamoxifen-alone arm.
- The ESR1-low plus proliferation-high metagene signatures were associated with more early DFS events, whereas proliferation-high best predicted greater risk for late events.
- A 5-metagene signature defined excellent prognosis for patients on tamoxifen alone, despite positive nodes.

CONCLUSIONS - III

- Differential benefit to CAF-T vs tamoxifen alone over 10 years of follow-up was seen in the low versus high risk groups, defined by 3 metagenes (ESR1, proliferation and TGFB).
- Chemotherapy is inferior to tamoxifen in the 3-metagene low risk group, but is superior to tamoxifen in the high risk subset.
- If validated, these signatures identify patients with excellent DFS despite positive nodes for endocrine therapy alone, and those for whom chemotherapy and/or biologics are also required.
ACKNOWLEDGEMENTS

- Our patients – who donated tumor samples 25+ years ago
- SWOG and other NCTN breast cancer investigators
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- Tina Rutschman (Loyola)
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