S4-5. Comparison of PAM50 risk of recurrence (ROR) score with oncotypexDx and IHC4 for predicting residual risk of RFS and distant RFS after endocrine therapy: A TransATAC study

• Dr. Dowsett has disclosed that he receives grant/research support from Astrazeneca. He has also disclosed that he is on the speakers bureau with Astrazeneca. He also disclosed that he receives other financial or material support from Astrazeneca for his legal advice.
• Dr. Lopez-Knowles has no relevant financial relationships to disclose.
• Ms. Sidhu has no relevant financial relationships to disclose.
• Ms. Pineda has no relevant financial relationships to disclose.
• Dr. Cowens has disclosed that he is an employee of Nanostring.
S4-5. Comparison of PAM50 risk of recurrence (ROR) score with oncotypeDx and IHC4 for predicting residual risk of RFS and distant RFS after endocrine therapy: A TransATAC study

- Dr. Ferree has disclosed that he is an employee of Nanostring.
- Dr. Storhoff has disclosed that he is an employee of Nanostring.
- Dr. Schaper has disclosed that he is an employee of Nanostring.
- Dr. Cuzick has disclosed that he receives grant/research support from Astrazeneca. He has also disclosed that he is on the speakers bureau with Astrazeneca. He also disclosed he is a consultant for Astrazeneca.
Comparison of PAM50 risk of recurrence (ROR) score with OncotypeDx and IHC4 for predicting residual risk of RFS and distant-(D)RFS after endocrine therapy: a TransATAC study.

DISCLOSURES

- Dr. Dowsett disclosed that he received grant support from and is on the speaker’s bureau, has received grant support and provided legal advice for AstraZeneca and has sponsored IHC4 in a NICE assessment.
- Drs. Lopez-Knowles, Sidhu and Sestak have no relevant financial relationships with commercial interests to disclose.
- Dr. Cowens, Ferree and Storhoff disclosed that they are employees of and shareholders in NanoString Technologies.
- Dr. Schaper disclosed that he is a consultant for NanoString Technologies.
- Dr. Cuzick disclosed that he received grant support from and is a consultant for AstraZeneca.
Comparison of PAM50 risk of recurrence (ROR) score with OncotypeDx and IHC4 for predicting residual risk of recurrence and distant recurrence after endocrine therapy: a TransATAC study.

Mitch Dowsett¹, Ivana Sestak⁴, Elena Lopez-Knowles¹, Kally Sidhu¹, J. Wayne Cowens², Sean Ferree², James Storhoff², Carl Schaper³, Jack Cuzick⁴

on behalf of the ATAC Trialists’ Group

¹. Royal Marsden Hospital, London, UK
². NanoString Technologies, Seattle, USA
³. Myraqa, Redwood City, USA
⁴. Wolfson Inst for Preventive Medicine, London, UK
Background

Estimating residual risk of recurrence in ER+ patients treated with endocrine therapy

GHI Oncotype Dx Recurrence Score (RS)

- Tamoxifen treatment
  - Node negative: Paik et al, NEJM, 2004, 351, 2817
- Tamoxifen or anastrozole (TransATAC)
  - Dowsett et al, JCO, 2010, 28, 1829
- Prediction improved with Clinical Treatment Score (CTS: nodes, grade, tumor size, age, treatment) (TransATAC)
  - Cuzick et al JCO, 2011, 29, 4273
  - Also Tang et al JCO, 2011, 29, 4365
Background

Estimating residual risk of recurrence in ER+ patients treated with endocrine therapy

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• Prediction improved with Clinical Treatment Score (CTS: nodes, grade, tumor size, age, treatment) (TransATAC)
  Cuzick et al JCO, 2011, 29, 4273
  Also Tang et al JCO, 2011, 29, 4365

IHC4 (ER, PgR, Ki67, HER2)

• Similar information to RS when added to CTS (TransATAC)
  Cuzick et al JCO, 2011, 29, 4273
Estimating residual risk of recurrence in ER+ patients treated with endocrine therapy

PAM50

- 50-gene test developed to identify the intrinsic breast cancer subtypes (luminal A/B, HER2-like, Basal-like)
- used to generate a Risk of Recurrence (ROR) Score
  - Parker et al, JCO, 2009, 27, 1160
  - Nielsen et al, CCR, 2010, 16, 5222
PAM50 by NanoString nCounter®

Extract RNA from FFPE tumor sample

Run RNA & PAM50 CodeSet on nCounter Analysis System

Capture patient expression profile

Determine Intrinsic Subtype through Pearson’s Correlation to Centroids

Calculate Risk of Recurrence (ROR) Score

\[ ROR = aR_{LumA} + bR_{LumB} + cR_{Her2e} + dR_{Basal} + eP + fT \]

Pearson’s correlation to centroids

Proliferation score (19 genes)

Tumor size
Overall goals

To determine whether PAM50 test adds prognostic information to clinical variables in TransATAC

To compare the performance of the ROR, RS and IHC4 Scores
Eligibility criteria

- HR+
- no chemotherapy
- tamoxifen or anastrozole alone
- RS available
- sufficient residual RNA for PAM50 analysis
- 10-year follow-up database
Samples analysed

ATAC\(^1\): 5216, HR+ tamoxifen or anastrozole

TransATAC\(^2\): 1782, centrally confirmed HR+

GHI RS\(^3\):
1231, evaluable with RS

1017, sufficient residual RNA*; 1007 pass PAM50 QA

PAM50 vs GHI

1007

IHC4 vs GHI RS\(^4\):
1125, with RS and IHC4

PAM50 vs GHI vs IHC4

940

* RNA extracted by GHI

Prospectively defined analysis plan

Does the ROR Score add prognostic information (Distant RFS) beyond the Clinical Treatment Score (CTS: includes nodes, grade, tumor size, age, treatment)?

(a) Primary analysis - in all patients

(b) Secondary analyses – in:

(i) node-negative patients
(ii) node-positive patients
(iii) HER2-negative patients

Statistics

Likelihood ratio test
Amount of information measured by change in likelihood ratio statistic, $\chi^2 (\Delta LR - \chi^2)$
Prospectively defined analysis plan

Does the ROR Score add prognostic information (Distant RFS) beyond the Clinical Treatment Score (CTS: includes nodes, grade, tumor size, age, treatment)?

(a) Primary analysis - in all patients
(b) Secondary analyses – in:
   (i) node-negative patients
   (ii) node-positive patients
   (iii) HER2-negative patients

Primary analysis (sequential)

Evaluation of 46-gene ROR Score
- excluding: BIRC5, MYBL2, GRB7, CCNB1
Does the ROR score add prognostic information for Distant Recurrence over and above the Clinical Treatment Score?

<table>
<thead>
<tr>
<th>Analyses</th>
<th>Population</th>
<th>No. Patients</th>
<th>ΔLR-χ²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>All Evaluable</td>
<td>1007</td>
<td>33.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Secondary</td>
<td>Node Negative</td>
<td>739</td>
<td>24.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Node Positive</td>
<td>268</td>
<td>9.3</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>HER2 Negative</td>
<td>888</td>
<td>28.0</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

ROR is significantly related to outcome for all populations tested.
Ten-year predicted risk of DR using ROR Score

Predicted 10-year risk of distant recurrence (%)

% of cases
Secondary analysis

• Comparison of the performance of ROR, RS, and IHC4 when added to Clinical Treatment Score (CTS: nodes, grade, tumor size, age, treatment)
Prognostic information beyond the Clinical Treatment Score for DR in all patients with all 3 measures (n=940)

Order of addition to model

Change in $LR^2$ Statistic

3rd Test Significant?

No

Yes

CTS is first test applied.

Graph shows information from 2nd & 3rd tests applied only
Prognostic information beyond the Clinical Treatment Score for DR in all patients with all 3 measures (n=940)

Graph shows information from 2nd & 3rd tests applied only
Prognostic information beyond the Clinical Treatment Score for DR in node negative patients with all 3 measures (n=683)

- ROR (PAM50)
- RS (Oncotype Dx)

Change in LRx² Statistic

Order of addition to model

<table>
<thead>
<tr>
<th>Change in LRx² Statistic</th>
<th>ROR → RS</th>
<th>RS → ROR</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.1</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>8.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3rd Test Significant?

No

Yes

CTS is first test applied.
Graph shows information from 2nd & 3rd tests applied only
Prognostic information beyond the Clinical Treatment Score for DR in node negative patients with all 3 measures (n=683)

CTS is first test applied.
Graph shows information from 2nd & 3rd tests applied only.
Prognostic information for DR in node negative patients (no Clinical Treatment Score)

Order of addition to model

Change LR^2 Statistic

ROR → RS

60.9

1.4

62.3

RS → ROR

28.2

34.1

62.3

2nd Test Significant?

No

Yes
Prognostic information for DR in node negative patients (no Clinical Treatment Score)

<table>
<thead>
<tr>
<th>Order of addition to model</th>
<th>Change LR$^2$ Statistic</th>
<th>2nd Test Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROR → RS</td>
<td>1.4</td>
<td>No</td>
</tr>
<tr>
<td>RS → ROR</td>
<td>34.1</td>
<td>Yes</td>
</tr>
<tr>
<td>ROR* → RS</td>
<td>1.6</td>
<td>No</td>
</tr>
<tr>
<td>RS → ROR*</td>
<td>28.5</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Legend:
- RS
- ROR (incl. tumor size)
- ROR* (excl. tumor size)
Secondary analysis: Comparing prognosis of risk groups for ROR and RS

- Estimated risk of 10-year DR for each patient using ROR and RS

- Defined Risk groups as predicted (in this dataset):
  - Low: < 10% Risk of 10-year DR
  - Intermediate: 10 - 20% Risk of 10-year DR
  - High: > 20% Risk of 10-year DR
Ten-year Risk Group Classification: ROR Score vs. RS in node negative patients (with Clinical Treatment Score)

<table>
<thead>
<tr>
<th>Score</th>
<th>H+L Groups</th>
<th>ROR</th>
<th>RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROR</td>
<td>572 (77%)</td>
<td>572 (77%)</td>
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</tr>
<tr>
<td>RS</td>
<td>538 (73%)</td>
<td>538 (73%)</td>
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</table>

<table>
<thead>
<tr>
<th>Score</th>
<th>H/L Hazard Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROR</td>
<td>7.16 [4.07 – 12.61]</td>
</tr>
<tr>
<td>RS</td>
<td>6.2 [3.3 – 11.5]</td>
</tr>
</tbody>
</table>
Ten-year Risk Group Classification: ROR Score vs. RS in node negative patients (no Clinical Treatment Score)

<table>
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<tr>
<th>Score</th>
<th>H+L Groups</th>
<th>ROR</th>
<th>RS</th>
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<tbody>
<tr>
<td>Low</td>
<td>428 (74%)</td>
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<tr>
<td>H/L</td>
<td>547</td>
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<td>434</td>
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<td>496 (67%)</td>
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<td>428</td>
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<td></td>
<td>Intermediate</td>
<td>192</td>
<td>243</td>
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<tr>
<td></td>
<td>H/L</td>
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</table>

Follow-up time (years)

Percent without distant recurrence (%)

<table>
<thead>
<tr>
<th>Score</th>
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</tr>
</thead>
<tbody>
<tr>
<td>ROR</td>
<td>7.20 [4.13 – 12.56]</td>
</tr>
<tr>
<td>RS</td>
<td>6.6 [3.5 – 12.5]</td>
</tr>
</tbody>
</table>

Patients in risk group

Score H+L Groups

ROR 547 (74%)
RS 496 (67%)
Secondary analyses

• Relationship of breast cancer intrinsic subtypes to 10yr risk of DR
Kaplan Meier curves of Luminal A vs. Luminal B by nodal status

Node Negative Patients
- Luminal A (529)
- Luminal B (176)

Node Positive Patients
- LumA, N1-3 (132)
- LumB, N1-3 (69)
- LumA, N ≥4 (31)
- LumB, N ≥4 (20)

Follow Up Time (Years)
- No Distance Recurrence (%)

HR = 4.78 (2.97 - 7.70)

Follow Up Time (Years)

HR (N1-3) = 2.20 (1.10 - 3.61)
HR (N ≥4) = 3.40 (1.60 - 7.22)
Alternate ROR Calculations

- All results presented using the 50-gene ROR score
- Additional primary analysis excluding 4 genes: BIRC5, MYBL2, GRB7, CCNB1
- The two versions of ROR had a correlation of 0.998.

<table>
<thead>
<tr>
<th>Analyses</th>
<th>Population</th>
<th>ROR50 ΔLR-χ²</th>
<th>ROR46 ΔLR-χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary</strong></td>
<td>All Evaluable (N=1007)</td>
<td>33.9</td>
<td>34.2</td>
</tr>
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<td><strong>Secondary</strong></td>
<td>Node Negative (N=739)</td>
<td>24.6</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>Node Positive (N=268)</td>
<td>9.3</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>HER2 Negative (N=888)</td>
<td>28.0</td>
<td>28.9</td>
</tr>
</tbody>
</table>

- Conclusions for the two versions were identical
Summary

• The PAM50 ROR score was significantly related to the probability of 10 year distant recurrence

• The ROR score added prognostic information beyond the Clinical Treatment Score in:
  • all patients
  • node-negative patients
  • node-positive patients
  • HER2-negative patients

• The breast cancer intrinsic subtypes, Luminal A and Luminal B, have significantly different outcomes when treated with endocrine therapy alone
Summary

- In this study the PAM50 ROR Score provided more prognostic information than Oncotype DX RS.
- Fewer patients were assigned to the intermediate risk group by PAM50 than by Oncotype DX RS, with equivalent or higher Hazard Ratios between the low and high risk groups.
- The ROR score provided at least as much prognostic information as IHC4.
Acknowledgements

ATAC trialists, pathologists and patients
ATAC Pathology sub-committee
ATAC Steering Committee
LATTE Steering Committee

Astrazeneca