The PAM50 Risk-of-Recurrence Score Predicts Risk for Late Distant Recurrence after Endocrine Therapy in Postmenopausal Women with Endocrine-Responsive Early Breast Cancer

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Abstract

**Purpose:** To assess the prognostic value of the PAM50 risk-of-recurrence (ROR) score on late distant recurrence (beyond 5 years after diagnosis and treatment) in a large cohort of postmenopausal, endocrine-responsive breast cancer patients.

**Experimental Design:** The PAM50 assay was performed on formalin-fixed paraffin-embedded whole-tumor sections of patients who had been enrolled in the Austrian Breast and Colorectal Cancer Study Group Trial 8 (ABCSG-8). RNA expression levels of the PAM50 genes were determined centrally using the nCounter Dx Analysis System. Late distant recurrence-free survival (DRFS) was analyzed using Cox models adjusted for clinical and pathologic parameters.

**Results:** PAM50 analysis was successfully performed in 1,246 ABCSG-8 patients. PAM50 ROR score and ROR-based risk groups provided significant additional prognostic information with respect to late DRFS compared with a combined score of clinical factors alone (ROR score: \(\Delta L_1 R^2 = 15.32, P < 0.001\); ROR-based risk groups: \(\Delta L_1 R^2 = 14.83, P < 0.001\)). Between years 5 and 15, we observed an absolute risk of distant recurrence of 2.4% in the low ROR-based risk group, as compared with 17.5% in the high ROR-based risk group. The DRFS differences according to the PAM50 ROR score were observed for both node-positive and node-negative disease.

**Conclusion:** PAM50 ROR score and ROR-based risk groups can differentiate patients with breast cancer with respect to their risk for late distant recurrence beyond what can be achieved with established clinicopathologic risk factors.

Introduction

More than two-thirds of breast cancers express estrogen receptor (ER) and/or progesterone receptor (PgR; ref. 1). These patients are candidates for treatment with drugs targeting ER signaling either by interfering with ligand binding (tamoxifen), blockade of estrogen biosynthesis (aromatase inhibitors or gonadotropin-releasing hormone analogs), or ER downregulation (fulvestrant). Adjunct treatment with tamoxifen reduces the risk-of-recurrence (ROR) in patients with ER-positive breast cancer over all time periods by 39% (1). Approximately 50% of recurrences in ER-positive disease will occur after the first 5 years beyond initial diagnosis and tamoxifen treatment (1). Mortality and recurrence risk vary over time according to molecular and clinical risk factors. In contrast with ER-negative tumors, which usually develop metastases early (mostly within 5 years) after initial diagnosis and treatment, the annual recurrence rates of ER-positive breast cancers are...
of aromatase inhibitor treatment. Ongoing trials such as can currently only be recommended to postmenopausal
ings suggest that extended aromatase inhibitor treatment reduce recurrence and breast cancer mortality during the
trials suggest that extended endocrine treatment can have been reported thus far (3–7). The results of these
extending endocrine therapy after 5 years of tamoxifen
unknown (2).

breast cancer–specific mortality beyond 5 years are currently patients with ER-positive tumors who are at highest risk for
cancers (2). Unfortunately, the molecular subpopulations of
are higher for ER-positive than for ER-negative breast can-
qup period, the annual breast cancer specific mortality rates
received 5 years of endocrine therapy. In that “late” follow-
cancer more than 5 years after diagnosis despite having
initially lower but persist beyond 5 years (2). Many patients
with ER-positive tumors will relapse and die from breast
cancer after diagnosis despite having received 5 years of endocrine therapy. In that “late” follow-
the annual breast cancer specific mortality rates
are higher for ER-positive than for ER-negative breast can-
cancer-specific mortality beyond 5 years are currently
unknown (2).

To reduce the risk of late recurrence and death (i.e., 5–10 years after diagnosis, or even later), several trials of
extending endocrine therapy after 5 years of tamoxifen
have been reported thus far (3–7). The results of these
tests suggest that extended endocrine treatment can
reduce recurrence and breast cancer mortality during the
second decade after diagnosis. Furthermore, these find-
ings suggest that extended aromatase inhibitor treatment
can currently only be recommended to postmenopausal
patients who had completed 5 years of tamoxifen, as there
are not yet sufficient data available for more than 5 years
of aromatase inhibitor treatment. Ongoing trials such as
ABCSD-16 (NCT00295620) are investigating the optimal
duration of extended adjuvant therapy.

Despite some clear-cut benefits, extended adjuvant ther-
apy comes with a considerable burden of toxicity for
patients and costs for society. Neither the optimal timing
and duration of extended adjuvant therapy nor the sub-
population of patients who will actually benefit are yet
identified, leaving clinicians and patients with uncertainty
about whether to continue endocrine therapy after the first 5
years. It would be extremely helpful to identify these
patients who are at particular persisting risk for late relapse
because they are most likely to benefit from extended
therapy. Conversely, it would be equally helpful to identify
patients at minimal risk of late distant recurrence as they
could be spared the side effects of an extended treatment
regimen from which they are unlikely to benefit. Thus, there
is currently a major unmet clinical need to accurately
identify breast cancer subpopulations that are either at high
or at low risk of late distant recurrence.

Multigene tests have recently been introduced for indi-
vidual risk assessment, and several of them have demon-
strated that they can add valuable prognostic information:
Oncotype DX (8), MammaPrint (9), Breast Cancer Index
(10), EndoPredict (11), and PAM50 (12–15) describe risk
scores based on the analysis of gene signatures established
and validated in clinical trials. Specific potential usefulness
for the prediction of late distant recurrence has been
reported in abstract form for PAM50 (16, 17), EndoPredict
(18), and Breast Cancer Index (17, 19).

The purpose of the present study is to evaluate in
patients from a large prospective trial whether the PAM50
ROR score is associated with late distant recurrence of
patients with hormone receptor–positive breast cancer
and therefore may be helpful in choosing the appropriate
candidates for extended therapy after 5 years of initial
docrine treatment.

Patients and Methods

Patients
All patients included in this study had participated in
the Austrian Breast and Colorectal cancer Study Group
Trial 8 (ABCSG-8). The study design, inclusion criteria,
and the main results of ABCSG-8 have been reported
elsewhere (20, 21). Between 1996 and 2004, 3,901 post-
menopausal women with hormone receptor–positive early-
stage breast cancer were randomized to receive either 5
years of adjuvant tamoxifen or tamoxifen for 2 years
followed by anastrozole for 3 years. Patients included in
ABCSG-8 did not receive neoadjuvant or adjuvant che-
motherapy, nor did any patient receive trastuzumab. The
sequence strategy of 2 years of tamoxifen followed by 3
years of anastrozole led to moderate outcome benefits
(20, 21).

The present study cohort consists of formalin-fixed par-
affin-embedded (FFPE) breast tumor tissue samples retro-
spectively collected and archived in the ABCSG tumor bank.
All tumor specimens were obtained at the time of surgery
before adjuvant therapy. Paraffin blocks were stored at
room temperature and were identifiable only by an identi-
fication number assigned to each patient at randomiza-
tion. Approval was obtained from the respective Ethics
Committee and an informed consent form was signed by
all participating patients. A detailed description of the
reconsent process including a CONSORT flow diagram is
reported elsewhere (15).

PAM50 assay description and ROR score calculation
PAM50 gene analyses were performed on the NanoString
counter device using the RNA extracted from pathologist-
reviewed, macrodissected FFPE sections (22, 23). Methods
followed prespecified and audited standard operating pro-
cedures within a Clinical Laboratory Improvement Amend-
ments (CLIA)-certified laboratory. Full details on conversion
of gene expression measurements into breast cancer intrinsic molecular subtypes and ROR scores for each case are shown elsewhere (15). Briefly, the ROR score was calculated using the test variables that include Pearson correlations with prototypical gene expression profiles for the four intrinsic subtypes (based on a 46-gene subset of the 50 genes), a proliferation score (mean expression of an 18-gene subset of the 50 genes), and pathologic tumor size (coded as 0 if ≤2 cm or 1 if >2 cm).

The test variables are multiplied by predefined weights, obtained originally from a Cox Proportional Hazards model during algorithm training on an independent patient cohort, summed, and then scaled to produce the ROR score that ranges from 0 to 100 according to the formula:

\[
ROR = 54.7690\times(-0.0067A + 0.4317B - 0.3172C + 0.4894D + 0.1981E + 0.1133F + 0.8826)
\]

where \(A\) = basal-like Pearson correlation, \(B\) = Her2-enriched Pearson correlation, \(C\) = luminal A Pearson correlation, \(D\) = luminal B Pearson correlation, \(E\) = proliferation score, and \(F\) = tumor size.

The ROR score was then categorized as low, intermediate, or high risk using prespecified ROR cutoffs as shown in Supplementary Table S1, incorporating information on the number of positive lymph nodes. These prespecified ROR score cutoffs were based on the transATAC data (14) with the intent of generating risk groups with 10-year probability of distant recurrence of <10%, 10–20%, and >20%.

Researchers generating the ROR scores and intrinsic molecular subtypes were blinded to both the test results and the clinical data.

**Statistical analysis**

All analyses were fully prespecified in a written plan and performed by statisticians working independently from those generating the gene expression and molecular subtype data. The primary endpoint was distant recurrence-free survival (DRFS), defined as the interval from randomization until distant recurrence or death due to breast cancer. Contralateral breast cancer, secondary malignancy and death due to causes other than breast cancer were treated as censoring events. Death due to breast cancer in which a recurrence had not been recorded was considered an event at the date of death.

A clinical linear predictor score was derived from the present data set as previously described (15). The clinical linear predictor is a linear combination of the standard clinical prognostic factors: age (≥65 vs. <65 years), grade (G2/GX vs. G1; G3 was an exclusion criterion in the original trial), tumor stage (T2/T3 vs. T1), lymph-node status (N1 vs. N0 and N2 vs. N0), and treatment (tamoxifen/anastrozole vs. tamoxifen only) according to CLP = \( \sum b_i z_i \), where \( z_i \) is the \( j \)th prognostic variable and \( b_i \) is the corresponding coefficient obtained by fitting the formula:

\[
\lambda(t) = \lambda_0(t) \exp \left( \sum_i z_i y_i \right)
\]

where \( \lambda(t) \) and \( \lambda_0(t) \) are the hazards and the baseline hazards, respectively.

Cox proportional hazards regression models were used to assess the effects of individual prognostic factors such as the clinical linear predictor, PAM50 ROR score, ROR score-derived risk groups, and intrinsic molecular subtypes; HRs with 95% confidence intervals (CI) were estimated. The log-likelihood test was used to test the hypotheses that the PAM50 ROR score, the ROR-based risk groups, and the intrinsic molecular subtype (including only Luminal A and Luminal B) added prognostic information beyond the clinical linear predictor score alone. Probabilities of 15-year DRFS with 95% CIs were estimated using the Kaplan–Meier method.

The analyses were performed on the basis of all the patients included in the trial and repeated for the node-negative and node-positive subgroups. A two-sided \( \alpha \) of 0.05 was used for all tests. All analyses were performed by two independent statisticians in parallel using SAS version 9.3 and R version 2.15.2.

**Results**

With a median follow-up time of 11 years, we observed 172 DRFS events (distant recurrence or death from breast cancer) in 1,478 patients. Seventeen of these were censored at the time of occurrence of a secondary malignancy before a DRFS event, resulting in 155 first DRFS events. Eighty-seven DRFS events occurred within the first 5 years (early DRFS events) and 68 DRFS events beyond 5 years (late DRFS events) after initial diagnosis and treatment (of which 50 events occurred between 5 and 10 years after diagnosis).

For further analysis, we excluded 87 patients with early DRFS events, 55 patients who died within the first 5 years without breast cancer or unknown breast cancer status, and 90 patients who had a secondary malignancy within the first 5 years. The prognostic impact of the PAM50 ROR score and ROR-based risk groups on late distant recurrence was evaluated in the remaining 1,246 patients beyond the first 5 years of initial diagnosis and treatment. Among those patients who had no DRFS events within the first 5 years after initial diagnosis, the low, intermediate, and high ROR-based risk groups comprise 460, 416, and 370 patients, respectively. Among node-negative patients, 448 were classified as low risk, 292 as intermediate risk, and 179 as high risk, whereas 12 of the node-positive patients fell into the low-risk category, 124 in the intermediate risk, and 191 in the high-risk group, respectively.

Kaplan–Meier analysis revealed that late DRFS is significantly different according to ROR-based risk groups in all patients (high vs. low: HR, 6.90; 95% CI, 3.08–15.45; \( P < 0.001 \); Fig. 1A) and in the node-negative (high vs. low: HR, 4.74; 95% CI, 1.89–11.87; \( P < 0.001 \); Fig. 1B) subgroup. In the node-positive patients, no late DRFS events were observed in the low ROR-based risk group and, therefore, the HRs between intermediate versus low and high versus low ROR-based risk groups cannot be calculated. Thus, we used the intermediate ROR-based risk group as reference (high vs. intermediate: HR, 3.15; 95% CI, 1.20–8.24; \( P = 0.02 \); Fig. 1C). The 15-year late
DRFS estimates according to the ROR-based risk groups are summarized in Table 1.

The prognostic impact of the PAM50 ROR score and ROR-based risk groups on late distant recurrence was further evaluated in Cox proportional hazards regression models adjusted for the clinical linear predictor score (Table 2). The clinical linear predictor score is a continuous variable that combines the standard clinical prognostic factors age, grade, tumor stage, lymph node status, and treatment as described in the Methods section. In these analyses, the PAM50 ROR score (HR, 1.03; 95% CI, 1.01–1.04; P < 0.001), and the ROR-based risk groups (intermediate vs. low: HR, 3.74; 95% CI, 2.38–6.90; P < 0.001) were associated with late DRFS independent of the clinical linear predictor score (Table 2). Similar results were obtained for the node-positive and node-negative subgroups (Table 2).

To test the hypotheses that the PAM50 ROR score or the ROR-based risk groups add prognostic information beyond the clinical linear predictor score alone, the log-likelihood test was used. The addition of the PAM50 ROR score to the clinical linear predictor score provides a highly significant further increase in prognostic information beyond 5 years (log-likelihood test: ΔLRχ² 15.23; P < 0.001; Table 3). Again, similar results were obtained in the node-negative and node-positive subgroups (Table 3). A highly significant increase in prognostic information beyond 5 years was also achieved by adding risk groups to the clinical linear predictor score (ΔLRχ² 14.83; P < 0.001; Table 3).
PAM50 can also be used to assign an intrinsic molecular subtype to all cases. Because all ABCSG-8 patients were hormone receptor–positive, most cases in the study population fall into the Luminal A (886, 71%) or Luminal B (331, 27%) categories. However, PAM50 reclassifies some of the cases into other molecular subtypes (29, 2%). Kaplan–Meier analysis showed that late DRFS is significantly different between Luminal A/B molecular subtypes in all patients (Fig. 2A) and in the node-negative subgroup (Fig. 2B). A similar difference in DRFS between

<table>
<thead>
<tr>
<th>Patients</th>
<th>Risk group</th>
<th>N</th>
<th>Events</th>
<th>15-year DRFS% (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>Low</td>
<td>460</td>
<td>7</td>
<td>97.6 (94.7–98.9)</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>416</td>
<td>23</td>
<td>90.9 (85.9–94.2)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>370</td>
<td>38</td>
<td>82.5 (74.8–88.1)</td>
</tr>
<tr>
<td>Node negative</td>
<td>Low</td>
<td>448</td>
<td>7</td>
<td>97.5 (94.6–98.9)</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>292</td>
<td>18</td>
<td>90.0 (83.6–94.0)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>179</td>
<td>13</td>
<td>85.8 (72.5–93.0)</td>
</tr>
<tr>
<td>Node positive</td>
<td>Low</td>
<td>12</td>
<td>0</td>
<td>100.0 (—)</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>124</td>
<td>5</td>
<td>93.5 (84.0–97.5)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>191</td>
<td>25</td>
<td>79.9 (70.0–86.8)</td>
</tr>
<tr>
<td>All</td>
<td>Luminal A</td>
<td>886</td>
<td>34</td>
<td>92.8 (88.6–95.1)</td>
</tr>
<tr>
<td></td>
<td>Luminal B</td>
<td>331</td>
<td>30</td>
<td>86.2 (78.2–91.3)</td>
</tr>
<tr>
<td>Node negative</td>
<td>Luminal A</td>
<td>656</td>
<td>20</td>
<td>94.7 (91.5–96.7)</td>
</tr>
<tr>
<td></td>
<td>Luminal B</td>
<td>240</td>
<td>16</td>
<td>88.7 (77.9–94.4)</td>
</tr>
<tr>
<td>Node positive</td>
<td>Luminal A</td>
<td>230</td>
<td>14</td>
<td>87.2 (76.9–93.1)</td>
</tr>
<tr>
<td></td>
<td>Luminal B</td>
<td>91</td>
<td>14</td>
<td>79.9 (66.6–88.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patients</th>
<th>Parameter</th>
<th>P</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>Clinical linear predictor score</td>
<td>&lt;0.001</td>
<td>2.14 (1.46–3.14)</td>
</tr>
<tr>
<td></td>
<td>PAM50 ROR score</td>
<td>&lt;0.001</td>
<td>1.03 (1.01–1.04)</td>
</tr>
<tr>
<td>Node negative</td>
<td>Clinical linear predictor score</td>
<td>0.06</td>
<td>1.84 (0.97–3.50)</td>
</tr>
<tr>
<td></td>
<td>PAM50 ROR score</td>
<td>0.007</td>
<td>1.02 (1.01–1.04)</td>
</tr>
<tr>
<td>Node positive</td>
<td>Clinical linear predictor score</td>
<td>0.11</td>
<td>1.85 (0.88–3.89)</td>
</tr>
<tr>
<td></td>
<td>PAM50 ROR score</td>
<td>0.003</td>
<td>1.03 (1.01–1.05)</td>
</tr>
<tr>
<td>All</td>
<td>Clinical linear predictor score</td>
<td>0.004</td>
<td>1.81 (1.21–2.71)</td>
</tr>
<tr>
<td></td>
<td>Intermediate versus low ROR</td>
<td>0.01</td>
<td>3.04 (1.29–7.19)</td>
</tr>
<tr>
<td></td>
<td>High versus low ROR</td>
<td>&lt;0.001</td>
<td>4.53 (1.92–10.71)</td>
</tr>
<tr>
<td>Node negative</td>
<td>Clinical linear predictor score</td>
<td>0.05</td>
<td>1.87 (0.99–3.52)</td>
</tr>
<tr>
<td></td>
<td>Intermediate versus low ROR</td>
<td>0.004</td>
<td>3.63 (1.51–8.74)</td>
</tr>
<tr>
<td></td>
<td>High versus low ROR</td>
<td>0.005</td>
<td>3.87 (1.51–9.90)</td>
</tr>
<tr>
<td>Node positive</td>
<td>Clinical Linear Predictor score</td>
<td>0.06</td>
<td>2.01 (0.97–4.17)</td>
</tr>
<tr>
<td></td>
<td>High versus intermediate ROR&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05</td>
<td>2.63 (0.99–6.99)</td>
</tr>
<tr>
<td>All</td>
<td>Clinical Linear Predictor score</td>
<td>&lt;0.001</td>
<td>2.36 (1.61–3.47)</td>
</tr>
<tr>
<td></td>
<td>Luminal B versus A</td>
<td>0.003</td>
<td>2.14 (1.30–3.50)</td>
</tr>
<tr>
<td>Node negative</td>
<td>Clinical Linear Predictor score</td>
<td>0.02</td>
<td>2.10 (1.10–4.01)</td>
</tr>
<tr>
<td></td>
<td>Luminal B versus A</td>
<td>0.04</td>
<td>2.03 (1.04–3.95)</td>
</tr>
<tr>
<td>Node positive</td>
<td>Clinical Linear Predictor score</td>
<td>0.03</td>
<td>2.33 (1.10–4.91)</td>
</tr>
<tr>
<td></td>
<td>Luminal B versus A</td>
<td>0.03</td>
<td>2.33 (1.11–4.92)</td>
</tr>
</tbody>
</table>

<sup>a</sup>No events in the low-risk group.
Luminal A and Luminal B cancers was observed within the first 5 years after diagnosis (Supplementary Fig. S1). In all the patients, the 15-year late DRFS estimates were 92.8% (95% CI, 89.6–95.1) for the Luminal A and 86.2% (95% CI, 78.2–91.3) for the Luminal B group (Table 1). The magnitude of prognostic differences between Luminal A and B molecular subtypes was less pronounced compared with the difference between ROR low- and high-risk groups. In the Cox model adjusted for the clinical linear predictor score, late DRFS was significantly longer in Luminal A patients compared with Luminal B (Luminal B vs. A: HR, 2.14, 95% CI, 1.30–3.50; \(P = 0.003\); Table 2). Moreover, Luminal A/B molecular subtypes add a significant amount of additional prognostic information to the clinical linear predictor score (\(\Delta LR^2\); 8.73; \(P = 0.003\); Table 3). This effect was also observed in node-negative and node-positive subgroups.

### Table 3. Additional prognostic information of the PAM50 ROR score, ROR-based risk groups, or luminal molecular subtypes expressed as difference in log-likelihood (\(\Delta LR^2\)) compared with the clinical linear predictor score alone

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients</th>
<th>(\Delta LR^2)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAM50 ROR score</td>
<td>All</td>
<td>15.32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Node negative</td>
<td>7.40</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Node positive</td>
<td>8.84</td>
<td>0.003</td>
</tr>
<tr>
<td>ROR-based risk groups</td>
<td>All</td>
<td>14.83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Node negative</td>
<td>11.96</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Node positive</td>
<td>5.92</td>
<td>0.05</td>
</tr>
<tr>
<td>Luminal molecular subtypes</td>
<td>All</td>
<td>8.73</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Node negative</td>
<td>4.17</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Node positive</td>
<td>4.82</td>
<td>0.03</td>
</tr>
</tbody>
</table>

### Discussion

In postmenopausal women with hormone receptor–positive early breast cancer, the PAM50 ROR score accurately predicted the individual risk of overall DRFS (14, 15). Moreover, the PAM50 ROR score added significant prognostic information beyond classic clinicopathologic disease characteristics, and this addition significantly increased the prognostic accuracy (14, 15). Furthermore, both ROR-defined risk groups and breast cancer intrinsic molecular subtypes demonstrated clinically meaningful differences with respect to their 5- and 10-year risk of distant recurrence. Because of the large study cohort of ABCSG-8, the preplanned application of the fully prespecified PAM50 classifier in its clinical test format, and in view of compatible results from applying the PAM50 classifier to the recently published ATAC trial, level 1 evidence is now reached according to Simon and colleagues for prospective/retrospective study.
designs (24). With this added prognostic information of the PAM50 ROR score, physicians can stratify patients into overall risk categories with different prognoses.

Because it is well known that breast cancer recurrence risk continues well beyond 5 years for luminal breast cancers, it would be of major clinical benefit to identify markers that address the risk of late recurrence. Beyond the issue of identifying a low-risk prognostic group that may not need chemotherapy, now established for several multigene classifiers (8–11, 15), identifying patients at persistent risk for late distant recurrence is an urgent unmet clinical need. Only a few studies so far have evaluated the risk of recurrence in women who completed 5 years of tamoxifen treatment and remain recurrence-free at 5 years (3–7).

The National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) MA.17 trial showed that 5 years of letrozole therapy in postmenopausal women with breast cancer who have completed 5 years of tamoxifen treatment improved disease-free survival (3). The benefit of extended endocrine therapy after 5 years of tamoxifen was confirmed by other trials, ABCSG-6a (4) and NSABP-33 (5). More recently, results from the ATLAS trial (6) and the aTTom trial (7) have shown that continuation of tamoxifen treatment out to 10 years, compared with stopping at 5 years reduced relative breast cancer mortality by about 50% during the second decade after diagnosis. On the basis of these results, extending adjuvant endocrine intervention may be an option to reduce the persistent long-term risks of late distant recurrence, yet is impractical to recommend for all patients, because of tolerability issues and economic limitations. Thus, the expected benefit of prolonged antihormonal treatment has to be weighed against toxicity (e.g., osteoporosis and fractures) and the individual likelihood of a late recurrence.

Large adjuvant clinical trials and particularly those with patients at limited overall risk of relapse, such as the ABCSG-8 cohort, are valuable resources for investigating factors that predict late recurrences. Several RNA-based multigene expression assays including Oncotype DX, EndoPredict, and MammaPrint have been developed to estimate the individual risk of recurrence of patients with breast cancer (8, 9, 11). In contrast with several other multigene tests (e.g., Oncotype DX and MammaPrint), EndoPredict and NanoString PAM50 can be performed in any qualified pathology laboratory. This eliminates the need for shipping tissue off site and delays in turnaround time.

It was reported by Dubsky and colleagues that the Endo-Predict assay predicts late DRFS in 1,702 patients with ER-positive/HER2-negative breast cancer from two adjuvant phase III trials (ABCSG-6 and 8) treated with 5 years of endocrine therapy (18). The EPClin score stratified 64% of patients at risk after 5 years into a low-risk subgroup with an absolute 1.8% risk of late distant recurrence at 10 years of follow-up. With the PAM50 ROR score, we obtained similar results based on a considerably longer follow-up—the absolute late distant recurrence risk at 10 years turned out to be an even lower 1.3% for the low risk group in the ABCSG-08 cohort.

In the TransATAC study, it has been shown that the ROR score, Oncotype DX, the IHC4 score, and the Breast Cancer Index provided significant prognostic information for early distant recurrence (0–5 years) beyond the clinical treatment score (CIS) in all patients (17). However, in years 5 to 10, only the ROR score ($D_{LR}^R < 15.7, P < 0.001$) and Breast Cancer Index ($D_{LR}^I 10.5, P < 0.05$) added substantial prognostic information beyond CIS for late distant recurrence (17).

Our study has several limitations. First, although we demonstrated that the PAM50 ROR score defines a group of patients with a high risk for late distant recurrence, we cannot prove that extended adjuvant endocrine therapy after 5 years of tamoxifen is beneficial and actually will improve individual outcomes in these patients. One possibility to evaluate the predictive value of the PAM50 ROR score would be a retrospective analysis of the tumor material of patients who had been enrolled in trials of extended endocrine therapy after 5 years of tamoxifen. Second, although the median follow-up of our study population is 11 years, the assay may be not predictive for very late recurrences. Finally, PAM50, like the other multigene expression assays, was not specifically developed in a late recurrence patient cohort.

For accurately predicting “low” risk of late relapse, however, these two limitations may be of minor importance. Although "predictiveness" of benefit of additional therapeutic intervention in high-risk situations ultimately requires prospective hypothesis testing in appropriate prospective clinical trials of these interventions (such as TailoRx NCT00310180 or MINDACT NCT00433589), for the determination of patient subgroups at low risk, absolute risk is a suitable parameter to demonstrate that any theoretical additional therapy could not improve outcomes further in a clinically relevant manner. For approximately one-third of ABCSG-8 patients in the PAM50 defined “low risk” category, we demonstrate an absolute cumulative risk of 2.4% for distant recurrence between years 5 and 15. Even if theoretical means existed to further improve this excellent outcome, it would require several thousands of patients in a prospective intervention trial to prove its efficacy, and most likely tolerability and health economic issues would exceed any minute absolute additional benefit of even a highly effective agent in such a low-risk group.

In summary, we have demonstrated in the large cohort of ABCSG-8 trial patients that PAM50 ROR score differentiates patients with postmenopausal, endocrine-responsive breast cancer with respect to their risk for late distant recurrence, in addition to and beyond established clinicopathologic risk factors.

Disclosure of Potential Conflicts of Interest
M. Filipits has honoraria from AstraZeneca, Eli Lilly and Company, Merck, Roche, and Sividon Diagnostics GmbH and is consultant/advisory board member of AstraZeneca, NanoString Technologies, and Sividon Diagnostics GmbH. T.O. Nielsen has ownership interest (including patents) in Bioclassifier LLC and is consultant/advisory board member of Bioclassifier LLC and NanoString Technologies. P. Dubsky has honoraria from Sividon Diagnostics GmbH and is consultant/advisory board member of Sividon Diagnostics GmbH, Genomic Health, and Agenda. C. Schaper is consultant/advisory board member of NanoString Technologies. S. Ferree has ownership interest (including patents) in NanoString Technologies. J.W. Cowens is Chief Medical Officer of and has ownership of...
interest (including patents) in NanoString Technologies. M. Gnant received commercial research grant from Roche, Sanofi-Aventis, Novartis, and Pfizer and has honoraria from Amgen, Pfizer, GlassxSmithKline, Bayer, Sandoz, AstraZeneca, Genomic Health, and NanoString Technologies. No potential conflicts of interest were disclosed by the other authors.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Filipits, R. Greil, C.F. Singer, P. Dubsky, C. Fešl, C. Schaper, J.W. Cowens, M. Gnant

References

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The PAM50 Risk-of-Recurrence Score Predicts Risk for Late Distant Recurrence after Endocrine Therapy in Postmenopausal Women with Endocrine-Responsive Early Breast Cancer

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