

Clinical Cancer Research



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

Martin Filipits, Torsten O. Nielsen, Margaretha Rudas, et al.

Clin Cancer Res Published OnlineFirst February 11, 2014.

Updated version Access the most recent version of this article at:
doi:[10.1158/1078-0432.CCR-13-1845](https://doi.org/10.1158/1078-0432.CCR-13-1845)

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.

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

Martin Filipits¹, Torsten O. Nielsen¹⁶, Margaretha Rudas², Richard Greil⁶, Herbert Stöger⁸, Raimund Jakesz³, Zsuzsanna Bago-Horvath², Otto Dietze⁷, Peter Regitnig⁹, Christine Gruber-Rossipal¹⁰, Elisabeth Müller-Holzner¹², Christian F. Singer⁴, Brigitte Mlineritsch⁶, Peter Dubsy³, Thomas Bauernhofer⁸, Michael Hubalek¹², Michael Knauer¹¹, Harald Trapl¹³, Christian Fesl⁵, Carl Schaper¹⁴, Sean Ferree¹⁵, Shuzhen Liu¹⁶, J. Wayne Cowens¹⁵, and Michael Gnant³, for the Austrian Breast and Colorectal Cancer Study Group

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 (ABCSCG-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 ABCSCG-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: $\Delta LR\chi^2$ 15.32, $P < 0.001$; ROR-based risk groups: $\Delta LR\chi^2$ 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. *Clin Cancer Res*; 1–8. ©2014 AACR.

Authors' Affiliations: Departments of ¹Medicine I, ²Pathology, and ³Surgery; ⁴Department of Obstetrics and Gynecology, Comprehensive Cancer Center Vienna, Medical University of Vienna; ⁵Austrian Breast and Colorectal Cancer Study Group, Vienna; Departments of ⁶Internal Medicine III and ⁷Pathology, Paracelsus Private Medical University, Salzburg; Departments of ⁸Internal Medicine and ⁹Pathology, Medical University of Graz, Graz; Departments of ¹⁰Pathology and ¹¹Surgery, Sisters of Charity Hospital and Cancer Center, Linz; ¹²Department of Obstetrics and Gynecology, Medical University of Innsbruck, Innsbruck; ¹³Department of Surgery, General Hospital Baden, Baden, Austria; ¹⁴Myraqa, Redwood Shores, California; ¹⁵NanoString Technologies, Seattle, Washington; and ¹⁶British Columbia Cancer Agency, Vancouver, British Columbia, Canada

Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Corresponding Author: Martin Filipits, Institute of Cancer Research, Department of Medicine I, Comprehensive Cancer Center Vienna, Medical University of Vienna, Borschkegasse 8a, A-1090 Vienna, Austria. Phone: 43-1-40160-57528; Fax: 43-1-40160-957500; E-mail: martin.filipits@meduniwien.ac.at

doi: 10.1158/1078-0432.CCR-13-1845

©2014 American Association for Cancer Research.

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). Adjuvant 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

Translational Relevance

In postmenopausal women with hormone receptor-positive breast cancer, the annual recurrence risk persists beyond the first 5 years of initial diagnosis and treatment. Extended endocrine therapy after 5 years of tamoxifen reduces the risk of late recurrence, but toxicity and cost must be considered. It would be of great value to differentiate patients at high versus low risk specifically of late relapse for clinical decision making. In the present study, we have shown that the PAM50 risk-of-recurrence (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. This ability to predict late recurrences may be used to identify patients with endocrine-responsive breast cancer who can be spared extended adjuvant therapy in the future.

initially lower but persist beyond 5 years (2). Many patients with ER-positive tumors will relapse and die from breast cancer more than 5 years after diagnosis despite having received 5 years of endocrine therapy. In that "late" follow-up period, the annual breast cancer specific mortality rates are higher for ER-positive than for ER-negative breast cancers (2). Unfortunately, the molecular subpopulations of patients with ER-positive tumors who are at highest risk for breast 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 trials suggest that extended endocrine treatment can reduce recurrence and breast cancer mortality during the second decade after diagnosis. Furthermore, these findings 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 ABCSG-16 (NCT00295620) are investigating the optimal duration of extended adjuvant therapy.

Despite some clear-cut benefits, extended adjuvant therapy 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 subpopulation 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 those 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 individual risk assessment, and several of them have demonstrated 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 endocrine 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 postmenopausal 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 chemotherapy, 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 paraffin-embedded (FFPE) breast tumor tissue samples retrospectively 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 identification number assigned to each patient at randomization. Approval was obtained from the respective Ethics Committee and an informed consent form was signed by all participating patients. A detailed description of the resection 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 nCounter device using the RNA extracted from pathologist-reviewed, macrodissected FFPE sections (22, 23). Methods followed prespecified and audited standard operating procedures within a Clinical Laboratory Improvement Amendments (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:

$$\text{ROR} = 54.7690^* (-0.0067^*A + 0.4317^*B - 0.3172^*C + 0.4894^*D + 0.1981^*E + 0.1133^*F + 0.8826)$$

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 $<10\%$, $10\text{--}20\%$, 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 $CLP = \sum b_j z_j$, where z_j is the j th prognostic variable and b_j is the corresponding coefficient obtained by fitting the formula:

$$\lambda(t) = \lambda_0(t) \exp\left(\sum_j z_j y_j\right)$$

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 α 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 into 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

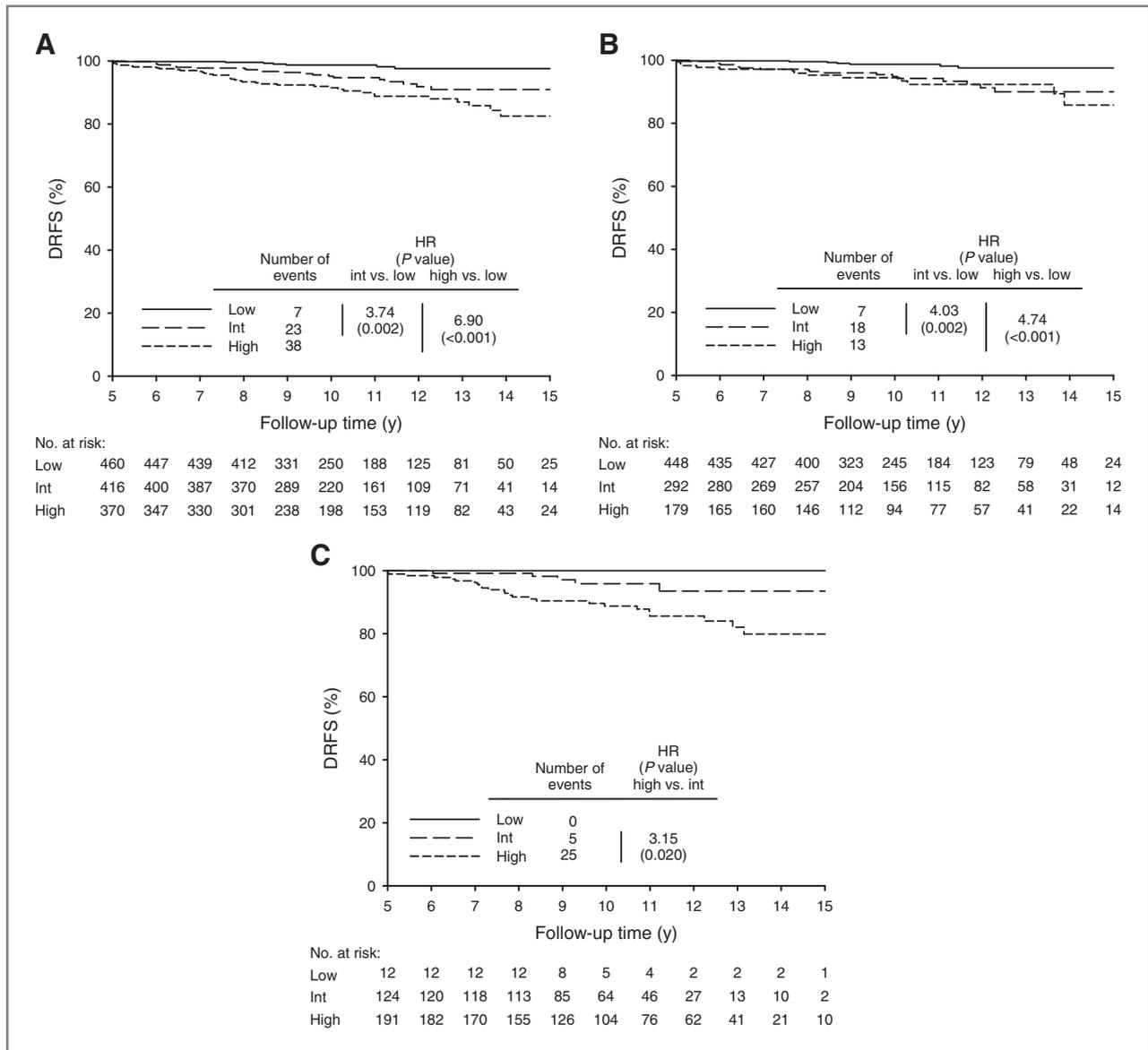


Figure 1. Kaplan-Meier plots of late DRFS according to ROR-based risk groups in all 1,246 patients with breast cancer (A) and node-negative (B) or node-positive (C) subgroups.

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, 3.04; 95% CI, 1.29–7.19; $P = 0.01$; high vs. low: HR, 4.53; 95% CI, 1.92–10.71; $P < 0.001$) 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: $\Delta LR\chi^2$ 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 ($\Delta LR\chi^2$ 14.83; $P < 0.001$; Table 3).

Table 1. Fifteen-year late DRFS estimates according to ROR-based risk groups and luminal molecular subtypes

Patients	Risk group	N	Events	15-year DRFS% (95% CI)
All	Low	460	7	97.6 (94.7–98.9)
	Intermediate	416	23	90.9 (85.9–94.2)
	High	370	38	82.5 (74.8–88.1)
Node negative	Low	448	7	97.5 (94.6–98.9)
	Intermediate	292	18	90.0 (83.6–94.0)
	High	179	13	85.8 (72.5–93.0)
Node positive	Low	12	0	100.0 (—)
	Intermediate	124	5	93.5 (84.0–97.5)
	High	191	25	79.9 (70.0–86.8)
All	Luminal A	886	34	92.8 (89.6–95.1)
	Luminal B	331	30	86.2 (78.2–91.3)
Node negative	Luminal A	656	20	94.7 (91.5–96.7)
	Luminal B	240	16	88.7 (77.9–94.4)
Node positive	Luminal A	230	14	87.2 (76.9–93.1)
	Luminal B	91	14	79.9 (66.6–88.3)

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 2. Cox proportional hazards regression models of late DRFS (beyond 5 years of initial diagnosis and treatment)

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

^aNo events in the low-risk group.

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

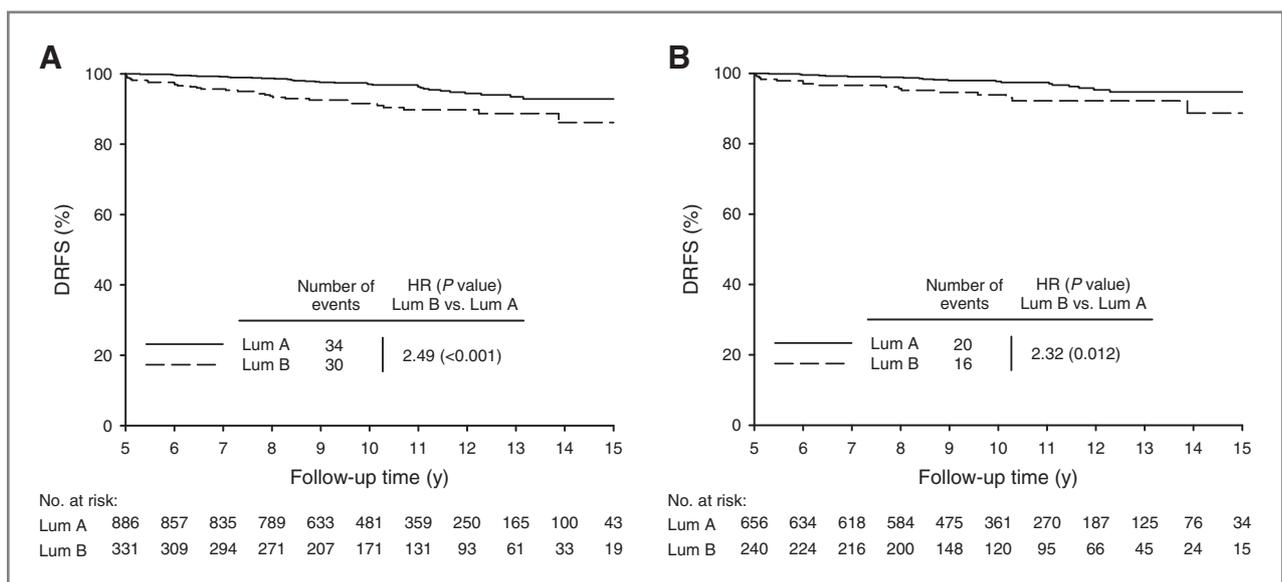
Variable	Patients	$\Delta LR\chi^2$	P
PAM50 ROR score	All	15.32	<0.001
	Node negative	7.40	0.007
	Node positive	8.94	0.003
ROR-based risk groups	All	14.83	<0.001
	Node negative	11.96	0.003
	Node positive	5.92	0.05
Luminal molecular subtypes	All	8.73	0.003
	Node negative	4.17	0.04
	Node positive	4.82	0.03

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 ($\Delta LR\chi^2$ 8.73; $P = 0.003$; Table 3). This effect was also observed in node-negative and node-positive subgroups.

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 pre-planned 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

**Figure 2.** Kaplan–Meier plots of late DRFS according to Luminal A/B molecular subtypes in all patients with breast cancer (A) and in the node-negative subgroup (B).

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 anti-hormonal 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 Dubsy and colleagues that the EndoPredict 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 (CTS) in all patients (17). However, in years 5 to 10, only the ROR score ($\Delta LR\chi^2$ 15.7, $P < 0.001$) and Breast Cancer Index ($\Delta LR\chi^2$ 10.5, $P < 0.05$) added substantial prognostic information beyond CTS 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. Dubsy has honoraria from Sividon Diagnostics GmbH and is consultant/advisory board member of Sividon Diagnostics GmbH, Genomic Health, and Agendia. 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

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, GlaxoSmithKline, Bayer, Sandoz, AstraZeneca, Genomic Health, and NanoString Technologies. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Concept and design: M. Filipits, C. Gruber-Rossipal, P. Dubsy, C. Schaper, J.W. Cowens, M. Gnant

Development of methodology: M. Filipits, T.O. Nielsen, C. Fesl, C. Schaper, S. Ferree, S. Liu, M. Gnant

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Filipits, T.O. Nielsen, M. Rudas, R. Greil, H. Stöger, R. Jakesz, Z. Bago-Horvath, O. Dietze, P. Regitnig, C. Gruber-Rossipal, E. Müller-Holzner, C.F. Singer, B. Mlineritsch, P. Dubsy, T. Bauernhofer, M. Hubalek, M. Knauer, H. Trapl, S. Ferree, S. Liu, M. Gnant

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

Writing, review, and/or revision of the manuscript: M. Filipits, T.O. Nielsen, M. Rudas, R. Greil, H. Stöger, R. Jakesz, Z. Bago-Horvath, P. Regitnig, C.F. Singer, P. Dubsy, T. Bauernhofer, M. Knauer, C. Fesl, C. Schaper, S. Ferree, S. Liu, J.W. Cowens, M. Gnant

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Filipits, T. Bauernhofer, M. Hubalek, C. Fesl, S. Liu, M. Gnant

Study supervision: M. Filipits, T.O. Nielsen, C.F. Singer, M. Gnant

Grant Support

The original clinical trial was in part supported by AstraZeneca. Additional follow-up, re-consent procedures, and database maintenance were supported by NanoString Technologies.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 4, 2013; revised October 8, 2013; accepted October 14, 2013; published OnlineFirst February 11, 2014.

References

- Davies C, Godwin J, Gray R, Clarke M, Cutter D, Darby S, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet* 2011;378:771–84.
- Yu KD, Wu J, Shen ZZ, Shao ZM. Hazard of breast cancer-specific mortality among women with estrogen receptor-positive breast cancer after five years from diagnosis: implication for extended endocrine therapy. *J Clin Endocrinol Metab* 2012;97:E2201–9.
- Goss PE, Ingle JN, Martino S, Robert NJ, Muss HB, Piccart MJ, et al. A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. *N Engl J Med* 2003;349:1793–802.
- Jakesz R, Greil R, Gnant M, Schmid M, Kwasny W, Kubista E, et al. Extended adjuvant therapy with anastrozole among postmenopausal breast cancer patients: results from the randomized Austrian Breast and Colorectal Cancer Study Group Trial 6a. *J Natl Cancer Inst* 2007;99:1845–53.
- Mamounas EP, Jeong JH, Wickerham DL, Smith RE, Ganz PA, Land SR, et al. Benefit from exemestane as extended adjuvant therapy after 5 years of adjuvant tamoxifen: intention-to-treat analysis of the National Surgical Adjuvant Breast and Bowel Project B-33 trial. *J Clin Oncol* 2008;26:1965–71.
- Davies C, Pan H, Godwin J, Gray R, Arriagada R, Raina V, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. *Lancet* 2013;381:805–16.
- Gray RG, Rea D, Handley K, Bowden SJ, Perry P, Earl HM, et al. aTTom: long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years in 6,953 women with early breast cancer. *J Clin Oncol* 2013;31(31suppl): abstr 5.
- Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004;351:2817–26.
- van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 2002;347:1999–2009.
- Sotiriou C, Wirapati P, Loi S, Harris A, Fox S, Smeds J, et al. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. *J Natl Cancer Inst* 2006;98:262–72.
- Filipits M, Rudas M, Jakesz R, Dubsy P, Fitzal F, Singer CF, et al. A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. *Clin Cancer Res* 2011;17:6012–20.
- Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. *Nature* 2000;406:747–52.
- Nielsen TO, Parker JS, Leung S, Voduc D, Ebbert M, Vickery T, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. *Clin Cancer Res* 2010;16:5222–32.
- Dowsett M, Sestak I, Lopez-Knowles E, Sidhu K, Dunbier A, Cowens JW, et al. Comparison of PAM50 risk of recurrence (ROR) score with Oncotype DX and IHC4 for predicting risk of recurrence and distant recurrence after endocrine therapy. *J Clin Oncol* 2013;31:2783–90.
- Gnant M, Filipits M, Greil R, Stoeger H, Rudas M, Bago-Horvath Z, et al. Predicting distant recurrence in receptor-positive breast cancer patients with limited clinicopathological risk: using the PAM50 Risk of Recurrence score in 1478 postmenopausal patients of the ABCSG-8 trial treated with adjuvant endocrine therapy alone. *Ann Oncol* 2013 doi: 10.1093/annonc/mdt494.
- Cuzick J, Sestak I, Ferree S, Cowens JW, Dowsett M. Prediction of late breast cancer recurrence by the ROR (PAM50) score in postmenopausal women in the TransATAC cohort. *Ann Oncol* 2012;23 Suppl. 9:75–6.
- Sestak I, Dowsett M, Sgroi D, Erlander M, Ferree S, Cowens JW, et al. Comparison of five different scores for the prediction of late recurrence for oestrogen receptor-positive breast cancer. *Ann Oncol* 2013;24 Suppl. 3:29.
- Dubsy P, Brase JC, Fisch K, Jakesz R, Singer CF, Greil R, et al. The EndoPredict score identifies late distant metastases in ER⁺/HER2⁻ breast cancer patients. *Cancer Res* 2012;72:1015.
- Sgroi DC, Sestak I, Cuzick J, Zhang Y, Schnabel CA, Erlander MG, et al. Comparative performance of Breast Cancer Index (BCI) vs. Oncotype Dx and IHC4 in the prediction of late recurrence in hormonal receptor-positive lymph node-negative breast cancer patients: a TransATAC study. *Cancer Res* 2012;72:92s–3s.
- Jakesz R, Jonat W, Gnant M, Mittlboeck M, Greil R, Tausch C, et al. Switching of postmenopausal women with endocrine-responsive early breast cancer to anastrozole after 2 years' adjuvant tamoxifen: combined results of ABCSG trial 8 and ARNO 95 trial. *Lancet* 2005;366:455–62.
- Dubsy PC, Jakesz R, Mlineritsch B, Postlberger S, Samonigg H, Kwasny W, et al. Tamoxifen and anastrozole as a sequencing strategy: a randomized controlled trial in postmenopausal patients with endocrine-responsive early breast cancer from the Austrian Breast and Colorectal Cancer Study Group. *J Clin Oncol* 2012;30:722–8.
- Geiss GK, Bumgarner RE, Birditt B, Dahl T, Dowidar N, Dunaway DL, et al. Direct multiplexed measurement of gene expression with color-coded probe pairs. *Nat Biotechnol* 2008;26:317–25.
- Kulkarni MM. Digital multiplexed gene expression analysis using the NanoString nCounter system. *Curr Protoc Mol Biol* 2011;Chapter 25: Unit25B 10.
- Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. *J Natl Cancer Inst* 2009;101:1446–52.