

## Pooled analysis of the prognostic relevance of progesterone receptor status in five German cohort studies

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**Abstract** The progesterone receptor (PR) has been increasingly well described as an important mediator of the pathogenesis and progression of breast cancer. The aim of this study was to assess the role of PR status as a prognostic factor in addition to other well-established prognostic factors. Data from five independent German breast cancer centers were pooled. A total of 7,965 breast cancer patients were included for whom information about their PR status was known, as well as other patient and tumor characteristics commonly used as prognostic factors.

Jessica Salmen and Julia Neugebauer have contributed equally to this study.

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Cox proportional hazards models were built to compare the predictive value of PR status in addition to age at diagnosis, tumor size, nodal status, grading, and estrogen receptor (ER) status. PR status significantly increased the accuracy of prognostic predictions with regard to overall survival, distant disease-free survival, and local recurrence-free survival. There were differences with regard to its prognostic value relative to subgroups such as nodal status, ER status, and grading. The prognostic value of PR status was greatest in patients with a positive nodal status, negative ER status, and low grading. The PR-status adds prognostic value in addition to ER status and should not be omitted from clinical routine testing. The significantly greater prognostic value in node-positive and high-grade tumors suggests a greater role in the progression of advanced and aggressive tumors.

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## Introduction

In recent decades, the treatment of patients with breast cancer and methods of predicting their prognosis and response to therapy have become increasingly based on molecular analyses. The aim is to individualize risk prediction and to tailor therapies individually to the patients and the tumors. Despite some major advances in treatment, approximately 17,000 patients still die of the disease in Germany every year [1]. Biomarkers used in clinical practice include tumor size, nodal status, grading, and estrogen receptor (ER) and HER2 receptor status as molecular markers [2]. However, other prognostic markers such as progesterone receptor (PR) status do not yet appear to have found a role in clinical practice. PR is usually used along with ER to assess the patient's responsiveness to hormone therapy. Some retrospective analyses of clinical cohorts have shown that PR is also useful for assessing the prognosis independently of other prognostic factors, including ER, and this has been discussed in the framework of prospective randomized studies [3, 4].

The progesterone (P4) pathway has been investigated in both healthy breast tissue and in breast cancer tumors. In efforts to investigate the pathogenesis of breast cancer, there have been a variety of studies on the role of P4 in the proliferation and morphogenesis of breast epithelial cells. Less is known about the role of P4 in breast cancer tumors [5].

P4 and PR appear to be involved in proliferative mechanisms in normal breast epithelial cells and in breast

cancer tumors. In healthy mammary tissue, PR-positive cells do not appear to be proliferative [6, 7], and proliferation seems to be mediated in a paracrine fashion. Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL) and receptor activator of nuclear factor- $\kappa$ B (RANK) are candidate factors for the mediation of the P4 effect through PR-positive luminal cells to RANK-positive basal breast epithelial cells [8–12]. In breast cancer tumors, P4 is able to mediate the proliferation of breast cancer cells in a way that appears to be more independent of paracrine signaling [13, 14] and plays an important role in the pathogenesis and progression of breast cancer.

Anti-hormonal therapies have been developed to a high level of clinical relevance in the treatment of breast cancer tumors [15–17], but the usefulness of P4 is still not yet established. P4 is not always co-expressed with ER, and loss of PR has been discussed to have an unfavorable effect on the prognosis in some clinical cohorts [18]. In addition, it has been noted that metastases from PR-positive tumors frequently lose PR expression after systemic spread and that this change in the tumor phenotype is associated with a more unfavorable prognosis [19, 20].

Most reports published to date have not had large enough sample sizes to address subgroup analyses and questions involving interactions, or have lacked other relevant patient and tumor characteristics such as grading, which we consider to be of specific importance. The aim of the present study was therefore to investigate the role of PR and its interactions with other commonly used prognostic factors in a large, pooled analysis of data from several breast cancer centers in Germany.

## Methods

The patients included in this retrospective study were recruited from cohort studies at five certified breast cancer centers in Germany (Erlangen, Freiburg, Heidelberg, Munich, Tübingen). Each of these centers contributed original data, which were pooled for the analysis. Thus, a total of 10,001 breast cancer cases were available, for which immunohistochemically assessed PR status was available. Study and patient characteristics for each study site are presented in supplementary Table 1. Further inclusion criteria for this analysis were histological proof of an invasive breast cancer, no evidence of distant metastasis at the time of the primary diagnosis, and information about overall survival, distant metastasis-free survival, and local recurrence-free survival. Applying these criteria, a total of 2,036 patients had to be excluded, resulting into a study population of 7,965 patients with primary, invasive breast cancer. Approval for the study was

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**Table 1** Patient and tumor characteristics and association with PR, showing mean and standard deviation (SD) for age at diagnosis and frequency and percentage for all other data

Characteristic	Value	PR- mean or N	PR- SD or %	PR+ mean or N	PR+ SD or %
Age at diagnosis (year)		56.6	12.5	57.4	12.0
pT	pT0/Tis <sup>a</sup>	62	2.3	52	1.0
	pT1	1,427	53.0	3,257	61.7
	pT2	972	36.1	1,659	31.5
	pT3	114	4.2	151	2.9
	pT4	115	4.3	156	3.0
Nodal Status	pN0	171	6.9	755	15.3
	pN+	1,254	50.4	3,449	69.8
Grading	G1	1,063	42.7	740	15.0
	G2	1,636	61.5	3,351	64.0
	G3	1,024	38.5	1,884	36.0
ER	ER–	1,644	61.3	444	8.4
	ER+	1,040	38.7	4,820	91.6
Chemotherapy	No	1,282	48.1	3,581	68.5
	Yes	1,384	51.9	1,645	31.5
Anti-hormone Therapy	No	1,798	68.1	2,055	39.7
	Yes	844	31.9	3,125	60.3

<sup>a</sup> pT0/Tis are patients who received a neoadjuvant chemotherapy. For those patients as well as for all other patients who received neoadjuvant chemotherapy molecular markers was assessed from the core biopsy

obtained from the local ethics committees at each university hospital.

#### Data collection

All of the participating breast centers are certified by the German Cancer Society and by the German Society for the Study of Breast Diseases (*Deutsche Gesellschaft für Senologie*). To obtain certification, a breast centre has to document each breast cancer case prospectively, including patient and tumor characteristics, treatment data, and epidemiological data. As part of the certification process, it is checked whether the treatment decisions taken are those recommended in accordance with the German guidelines for the treatment of breast cancer. This ensures fairly homogeneous treatment of breast cancer patients across several institutions. Follow-up information has to be provided for up to 10 years after the primary diagnosis. In addition, all histological data have to be documented—such as tumor size, axillary lymph-node status, grading, ER status, PR status, and HER2/neu status. As part of the continuous certification process, the quality of the data is audited and re-audited annually. Data obtained from this process were used in the analysis presented here.

PR status was assessed according to the centers' protocols in routine clinical immunohistochemical protocols. The interpretation of whether or not a stain was positive

was left to the discretion of the investigators at each study site. However, the patients were recruited at a time when 10 % was the cut-off value for positivity. In case neoadjuvant chemotherapy was given, hormone receptor assessment as well as grading had to be available for the pretherapeutic core biopsy.

#### Statistical considerations

The primary objective was to study whether PR status information is a prognostic factor for overall survival (OS), distant metastasis-free survival (DMFS), and local recurrence-free survival (LRFS) in addition to well-known prognostic factors. For this purpose, Cox proportional hazards regression analyses as described below were carried out with OS, DMFS, and LRFS, respectively, as outcome and the following predictors: PR (positive vs. negative), age at diagnosis (continuous), pT (ordinal), ER (positive vs. negative), grading (ordinal), nodal status (positive vs. negative), chemotherapy (yes vs. no), and anti-hormone therapy (yes vs. no).

Patients with missing outcome and patients with missing PR information were excluded. Missing predictor values were imputed using single “best guesses” (median value of continuous or integer predictors, the most common value of categorical or ordinal predictors) based on non-missing data across all subjects. Continuous predictors were used as

natural cubic spline functions to describe non-linear effects [21]. The number of degrees of freedom (between one and four) of each predictor was determined by first fitting several simple cubic spline Cox regression models which differ from each other by the number of degrees of freedom, and then choosing the number of degrees of freedom which optimizes the Akaike information criterion (AIC). The AIC was used because it measures goodness of fit and also takes over-fitting into account by penalizing complex models.

The main survival analysis started with a Cox regression model with the well-known prognostic factors described above but without PR (*based model*). Next, another Cox model was fitted containing PR, the prognostic factors from the base model and the interactions of these prognostic factors with PR (*full model*). Both models were compared using the likelihood ratio test. A significant test results means that PR information improved the survival prognosis additionally to the considered well-known prognostic factors either across all patients or at least within one of the subgroups defined by the interaction terms. In case of a non-significant result, no further analyses were carried out to avoid false-positive results. If, however, the  $p$  value was significant, then the following variable selection procedure was performed to identify predictors which are associated with PR regarding survival: 1,000 bootstrap samples of the same size as the original dataset were taken with replacement from the original dataset. On each bootstrap sample, the full Cox model as defined above was fitted. A backward stepwise variable selection which kept all the predictors of the base model was carried out to obtain the best model in accordance with the AIC. The retained variables from each bootstrap sample were recorded, and a final variable selection was made by applying a procedure proposed by Sauerbrei and Schumacher [22]. In this procedure, the most frequently selected (>70 %) variables were chosen, and, to address correlation among variables, the variable with the larger frequency out of each highly frequent variable pair (>90 %) was chosen. A Cox regression model with these finally selected variables was fitted to the original dataset (the final model). Due to the selection conditions, the final model necessarily contained all the predictors of the base model and additionally, it possibly contained PR but no interaction term or possibly PR and at least one interaction term. Repetitive variable selections were carried out to get a stable stepwise regression result [23].

Hazard ratios (HR) with 95 % confidence intervals based on the final model were calculated. An overall HR representing the average prognostic effect of PR across all subgroups as well as HRs representing the average prognostic effect of PR within subgroups was shown. The fact that the study was multi-centric was taken into account by stratifying the models by study center, i.e., different baseline hazard functions were estimated for each study center.

Interesting findings were illustrated using Kaplan–Meier curves.

The proportional hazards assumptions were checked using the method of Grambsch and Therneau [24]. If the proportional hazards assumptions had not been fulfilled, the analysis was repeated separately for survival time up to 5 years and from 5 years on.

The performance of the final Cox models in terms of discrimination and calibration (“goodness of fit”) was measured with the area under the receiver operator curve (AUC) for survival data [25] and the modified Hosmer–Lemeshow statistic for survival data proposed by Gronnesby and Borgan [26]. The AUC ranges from 0.5 (no discrimination at all) to 1 (perfect discrimination at any time point between patients with event already and patients without event then). Following Gronnesby and Borgan, the observed number of events and the model-based estimated number of events within deciles of predicted risk were compared using a goodness of fit  $\chi^2$  test. A large  $p$  value indicates a satisfactory calibration.

All of the tests were two sided, and a  $p$  value <0.05 was regarded as statistically significant. Calculations were carried out using the R system for statistical computing (version 2.13.1; R Development Core Team, Vienna, Austria, 2011).

## Results

A total of 7,965 patients were included in the analysis. The percentage of missing values in each variable was below 2 % except of grading (6.7 %). The missing values were imputed as described above. At the time of diagnosis, their average age was 57 years ( $\pm 12$  years). The PR status was positive in 5,275 patients (66.2 %), and the ER status was positive in 5,860 (73.7 %) patients. Most of the patients had an early tumor stage and were node negative, with a grade of two. Chemotherapy was administered in 38.4 % of the cases ( $n = 3,969$ ). Patients with a positive PR status were older and more often had a lower tumor stage, a lower grade, and more often a positive ER status. Detailed patient characteristics are shown in Table 1. The median follow-up time was 5.1 years for OS, and 4.1 years for DMFS and LRFS, respectively.

## Survival analyses

The survival analyses had to be carried out separately for the first 5 years and the second 5 years of the follow-up period for overall survival and for distant metastasis-free survival, as the proportional hazards assumptions were fulfilled for the whole follow-up period only for local recurrence-free survival. The preliminary survival analysis

**Table 2** Bootstrap-based Cox regression analyses<sup>a</sup>, showing inclusion frequencies<sup>b</sup> (per cent) in 1,000 bootstrap samples and final selection (yes/no) according to pre-specified criteria

Predictor <sup>c</sup>	OS 0–5 y %	Yes	DMFS 0–5 y %	Yes	DMFS 5–10 y %	Yes	LRFS 0–10 y %	Yes
PR	100.0	Yes	100.0	Yes	100.0	Yes	99.9	Yes
PR × Age	88.9	Yes	30.5	No	31.7	No	74.7	Yes
PR × pN	74.9	Yes	47.2	No	58.9	No	88.0	Yes
PR × Grading	49.5	No	85.9	Yes	73.4	Yes	34.0	No
PR × ER	35.5	No	89.8	Yes	76.9	Yes	24.6	No
PR × pT	24.0	No	31.9	No	19.4	No	41.1	No
PR × Chemotherapy	21.5	No	19.5	No	39.5	No	48.4	No
PR × Antihormonal therapy	20.5	No	26.3	No	41.9	No	34.1	No

<sup>a</sup> There were no selection procedures for OS at the second period (5–10 years) because of non-significant results of the likelihood ratio tests comparing the base model with the full interaction model

<sup>b</sup> Inclusion frequency may be regarded as a measure of variable importance

<sup>c</sup> The other predictors of the full Cox model (i.e., age, pN, ..., antihormonal therapy) were kept in all bootstrap samples

**Table 3** Final Cox regression models, showing subgroup specific as well as overall hazard ratio (HR) for PR (positive vs negative) and associated 95 % confidence interval

Predictor	OS (0–5 years)	DMFS (0–5 years)	DMFS (5–10 years)	LRFS (0–10 years)	
Age <sup>a</sup>	low (42 years)	0.58 (0.43, 0.79)	– <sup>b</sup>	– <sup>b</sup>	1.05 (0.79, 1.40)
	middle (56 years)	0.61 (0.48, 0.76)	– <sup>b</sup>	– <sup>b</sup>	0.78 (0.61, 1.00)
	high (74 years)	0.86 (0.69, 1.07)	– <sup>b</sup>	– <sup>b</sup>	0.74 (0.52, 1.06)
Nodal status	pN0	0.71 (0.53, 0.95)	– <sup>b</sup>	– <sup>b</sup>	0.96 (0.72, 1.29)
	pN+	0.52 (0.40, 0.66)	– <sup>b</sup>	– <sup>b</sup>	0.63 (0.47, 0.85)
Grading	G1	– <sup>b</sup>	0.34 (0.22, 0.54)	0.42 (0.17, 1.03)	– <sup>b</sup>
	G2	– <sup>b</sup>	0.49 (0.39, 0.63)	0.79 (0.50, 1.25)	– <sup>b</sup>
	G3	– <sup>b</sup>	0.70 (0.53, 0.93)	1.49 (0.77, 2.91)	– <sup>b</sup>
ER	ER–	– <sup>b</sup>	0.37 (0.25, 0.56)	1.30 (0.59, 2.89)	– <sup>b</sup>
	ER+	– <sup>b</sup>	0.65 (0.52, 0.81)	0.48 (0.32, 0.72)	– <sup>b</sup>
Overall	–	0.61 (0.48, 0.76)	0.49 (0.39, 0.63)	0.79 (0.50, 1.25)	0.78 (0.61, 1.00)

<sup>a</sup> Age was regarded as continuous predictor. It was evaluated at first decile (“low”), median (“middle”), and 9th decile (“high”)

<sup>b</sup> There are no subgroup specific HR for this predictor, because the corresponding interaction term was dropped during the variable selection process

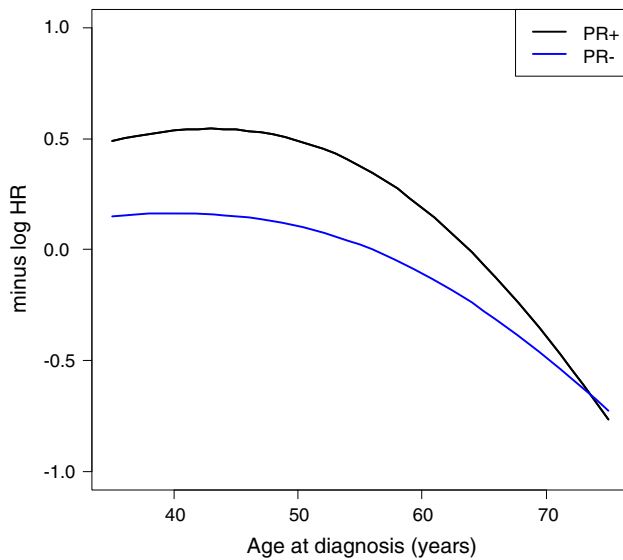
showed that the continuous predictor age was fitted best as a cubic spline variable with 2, 2, and 3 degrees of freedom for OS, DMFS, and LRFS, respectively. The performance of the final models, which were discussed below, seemed to be quite good with AUC values between 0.72 and 0.77 for OS and DMFS, and 0.64 for LRFS. The *p*-values of the Hosmer–Lemeshow tests ranged from 0.22 to 0.70 indicating satisfactory calibrations.

#### Overall survival

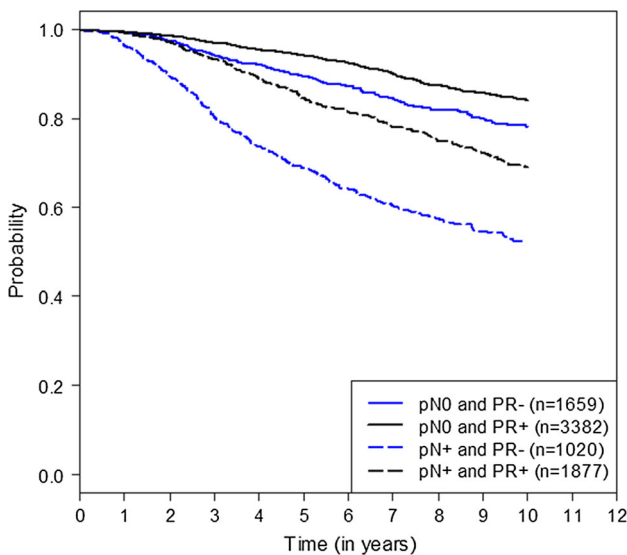
In relation to the first 5 years of follow-up, the multiple Cox survival analysis showed that the prognosis can be

improved overall by taking PR into account ( $p < 0.001$ , likelihood ratio test comparing the base model with the full interaction model). The most frequent selected interactions at the repetitive variable selection process were PR by age (89 %) and PR by nodal status (75 %) resulting in a final Cox regression model with PR, these interactions and the prognostic factors from Table 1. All other interaction terms were selected in less than 60 % of all bootstrap samples. The percent frequencies are listed in Table 2. These frequency values may be regarded as a measure of variable importance.

On average—i.e., without examining specific subgroups—patients with PR-positive tumors had a better

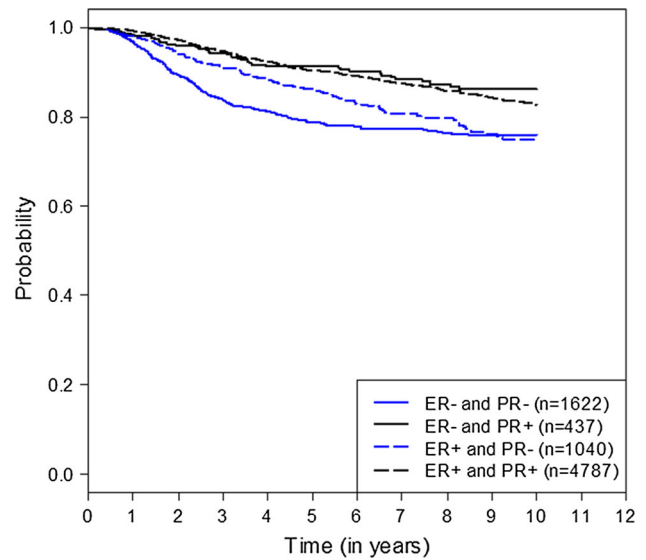


**Fig. 1** Overall survival prognosis in patients with progesterone negative and progesterone-positive tumors relative to their age at diagnosis. The results (in terms of log hazard ratio with PR- and 56 years as reference) are based on the final Cox regression model with age as cubic spline function adjusted for the prognostic factors in Table 1. The higher the value on the y-axis, the better the survival prognosis. The log hazard ratio (HR) for PR-positive versus PR-negative for patients with a specific age can be read by fixing the age value and subtracting the associated y-values on the *black* and *blue* curves

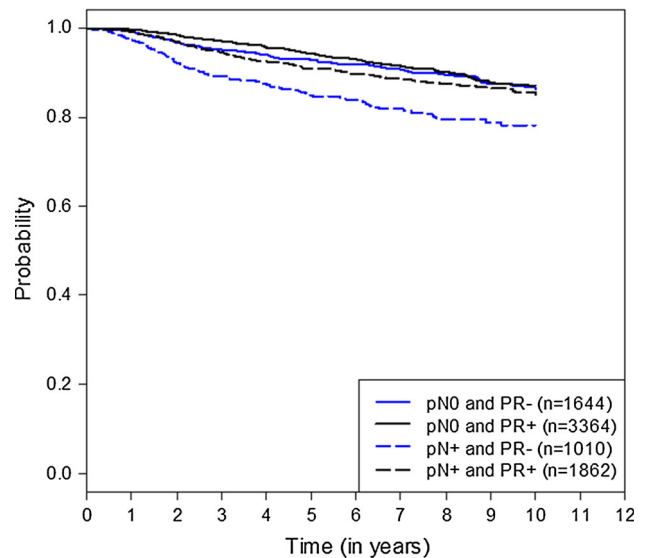


**Fig. 2** Overall survival. Showing Kaplan–Meier curve for PR and nodal status

overall survival prognosis than PR-negative patients (HR 0.61; 95 % CI, 0.48–0.76). However, the benefit differed between patient groups. Younger patients and node-positive patients benefited more than their counterparts (Table 3, Figs. 1, 2). We could not show that PR has prognostic value after 5 years of follow-up ( $p = 0.06$ , likelihood ratio test).



**Fig. 3** Distant metastasis-free survival. Showing Kaplan–Meier curve for ER and PR



**Fig. 4** Local recurrence-free survival. Showing Kaplan–Meier curve for pN and ER

Distant metastasis-free survival

PR proved to be a significant additional predictor both in the first period and in the second period, according to likelihood ratio tests comparing base models and full models ( $p < 0.00001$  and  $p = 0.02$ ). An overall effect could be shown in the first period (HR 0.49; 95 % CI, 0.39–0.63) but not in the second period (HR 0.79; 95 % CI, 0.51–1.25). In both periods, the prognostic effect differed between patient groups defined by grading and nodal status. (Tables 2, 3). A higher grading is associated with decreasing benefit for patients with PR-positive tumors

compared to PR-negative patients throughout the whole follow-up time. Within the first five years, the survival difference between PR-positive and PR-negative patients is larger in ER-negative patients than in ER-positive patients. After five years, however, there were no differences anymore in ER-positive patients, whereas the differences increased in ER-positive patients (Table 3, Fig. 3).

#### Local recurrence-free survival

PR significantly improved the prognosis throughout the total observation time ( $p = 0.02$ , likelihood ratio test): Young patients with PR-negative tumors seemed to live longer local recurrence free than young patients with PR-positive tumors. This effect might be reversed with increasing age. A relevant prognostic effect of PR information could be shown in nodal positive but not in nodal-negative patients (Table 3, Fig. 4).

## Discussion

This retrospective study shows that data on PR status can improve the prediction of each survival outcome (crude OS, DMFS, and LRFs) in addition to the commonly established prognostic factors. Specifically, the effect of PR status on prognosis was different among the subgroups of nodal status, grade, and ER status. It appeared that the protective prognostic effect of PR status was greatest in patients with node-positive disease, a low-grade tumor, and negative ER status.

A large study by Bardou et al. reported a better disease-free survival, with a HR of 0.62 (95 % CI, 0.49–0.80) and a better overall survival with a HR of 0.67 (95 % CI, 0.54–0.82) for patients with a positive PR status [3]. Liu et al. reported a HR of 0.76 (95 % CI, 0.59–0.98), but in a group that only included ER-positive patients [27]. The present study found HRs that were lower than those in Bardou et al., but the HR for ER-positive patients was very similar to that in Liu et al. Similar effects have also been reported in other studies [28–30]. Some of these studies analyzed survival in subgroups relative to the interaction between ER status and PR status [3, 29, 30], but without taking further interactions into consideration. The other studies did not analyze for any interactions [27, 28].

The present analysis was modeled in relation to the question of whether or not the addition of PR status, and its interaction terms is able to improve the prediction of the prognosis over and above the commonly established factors, including ER. In addition to an interaction with ER, interactions with nodal status and grading were identified, as well as evidence suggesting that the progression of breast cancer is a process, for the understanding of which

more—probably still unknown—factors have to be taken into consideration.

There have been several studies on the involvement of P4 and PR in the proliferation of benign, premalignant, and malignant breast cells [5]. Proliferation is part of the grading assessment, which showed an interaction with PR status relative to the prognosis in the present study. As mentioned, PR-positive benign breast epithelial cells are not highly proliferative [6, 7], but during carcinogenesis, the proportion of proliferating PR-positive cells appears to increase [31–34], and this has been linked to an increased risk of progression to malignant breast cancer cells [35]. In invasive breast cancer tumors, there are both PR-positive cells with a high grading or proliferation and PR-positive cells with a low grading or proliferation. In some breast tumors, therefore, it appears that there is a link between PR signaling and proliferation, while in others there is not. In preclinical models, the progesterone–PR pathway appears to lead to a transient proliferation of breast cancer cells, although resulting in subsequent cell cycle arrest [5, 35–38]. In some breast cancers, however, this pathway is considerably altered [39].

In the present study, PR status was best able to differentiate prognostic groups in low-grade tumors. This effect appeared to become smaller from low-grade to high-grade tumors. The HR (DMFS) for low-grade tumors was 0.34 (95 % CI, 0.22–0.54); this increased to 0.70 (95 % CI, 0.53–0.93). Grading and proliferation play an important role in the differentiation of intrinsic molecular types of breast cancer [40–42]. Proliferation as assessed by Ki-67, for example, is helpful for distinguishing between luminal A and luminal B breast cancers. Luminal B breast cancers have been reported to show a 53 % rate of grade three tumors, whereas only 19 % of luminal A breast cancers were grade three [40]. Similarly, in the present study only 41 % of tumors with a high grade were PR-positive, in comparison with 81 % of grade one tumors. It appears that the loss of the PR has a relatively greater impact on the prognosis in this subgroup than in tumors with a high grade, in which other molecular pathways may also contribute to the unfavorable outcome.

The present study has several strengths and weaknesses. Strengths include its large sample size and multicenter nature. The findings were obtained after a stratified analysis of data from the participating study centers and were found consistently for the several outcomes of OS, DMFS, and LFRS. However, the multicenter design also involves some weaknesses. The study is retrospective, and the data were therefore collected heterogeneously in all the participating hospitals (detailed data in supplementary Table 1). This might be of particular importance in relation to immunohistochemical assessment not only of PR, but also ER. ER and PR status were defined in the study according to the local

assessment at the participating study site, but a cut-off value of 10 % positively stained cells was usually used to determine the hormone receptor status. It should be borne in mind that other studies have reported that up to 20 % of assessments were inaccurate [43]. In addition, the cut-off value for hormone responsiveness is currently regarded as lying at 1 % rather than 10 %, further limiting the generalizability of the data. With regard to molecular subgroups, it should be pointed out that no proliferation markers or HER2 status were available for all study centers. It has to be kept in mind that the analysis was performed in a heterogeneously treated patient population. Therefore, the described effects are most probably the result of both, a prognostic effect and an effect which translates into prognosis due to different effectiveness of chemotherapy and anti-hormone therapy. However, anti-hormone therapy and chemotherapy were both taken into account in our analysis. As no interaction variables between PR status and therapy were significant, it can be assumed that the effects for the prediction of prognosis are similar in treated and untreated patients.

## Conclusions

The study shows that data on the PR status of patients with breast cancer are capable of improving prognostic predictions over and above other commonly used prognostic factors. However, the value of the PR status appeared to differ in different subgroups including nodal status, grading, and ER status. Assessment of the PR status should be a mandatory part of assessing the prognosis of breast cancer patients.

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