

## High Progesterone Receptor Expression Correlates to the Effect of Adjuvant Tamoxifen in Premenopausal Breast Cancer Patients

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**Abstract Purpose:** Tamoxifen has long been the drug of choice in adjuvant endocrine therapy of steroid hormone receptor–positive breast cancer, and it still remains important due to its well-documented beneficial effect. Hormone receptor status is often reported as “positive” or “negative” using 10% positive nuclei as a cutoff. In this study, we aimed to assess whether a further subclassification of hormone receptor status could enhance the treatment predictive value.

**Experimental Design:** The immunohistochemical expression of estrogen receptor (ER) and progesterone receptor (PR) was quantified in tissue microarrays with tumors from 500 premenopausal breast cancer patients previously included in a randomized trial of adjuvant tamoxifen compared with an untreated control group.

**Results:** Our findings show a gradually increasing tamoxifen effect in tumors with >10% ER-positive nuclei. However, when analyzing tamoxifen response according to various PR fractions, we found that it was primarily patients with tumors showing >75% PR-positive nuclei that responded to tamoxifen treatment, with an improved recurrence-free [relative risk, 0.42 (0.25–0.70);  $P = 0.001$ ] as well as overall [relative risk, 0.49 (0.28–0.84);  $P = 0.010$ ] survival.

**Conclusions:** Adjuvant tamoxifen improved recurrence-free and overall survival for premenopausal patients with tumors showing >75% PR-positive nuclei. No effect could be shown in tumors with fewer PR-positive nuclei. The PR was a stronger predictor of treatment response than the ER. Based on these findings, we suggest the implementation of a fractionated rather than dichotomized immunohistochemical evaluation of hormone receptors in clinical practice, possibly with greater emphasis on the PR than the ER.

The selective estrogen receptor modulator (SERM) tamoxifen has long been the most important adjuvant treatment for both premenopausal and postmenopausal hormone receptor–positive breast cancer of all stages (1). Although studies of aromatase inhibitors show promising results (2–5), tamoxifen still remains the drug of choice due to its well-documented effect. However, because treatment is not without side effects and all tumors do not respond, it is important to improve the prediction of treatment response. In the past decade, immunohistochemical assays have replaced cytosolic assays and

studies comparing the predictive value of the two methods have found immunohistochemistry to be an equally or more reliable alternative (6–13). Several studies have suggested that the level of hormone receptor content is of importance, and some have suggested that the progesterone receptor is a better predictor than the estrogen receptor (14–20). However, despite the prevailing notion that there may be predictive information to be gained, no studies have, to our knowledge, investigated the value of a fractionated assessment of hormone receptors using immunohistochemistry. In clinical practice, tumors with >10% ER-positive and/or PR-positive nuclei are often considered hormone receptor positive and these patients are subjected to endocrine therapy. The purpose of this study was to evaluate the predictive effect of ER and PR levels, defined as various fractions of positive cells. To properly evaluate the benefit of a given treatment, a randomized trial with an untreated control arm is an optimal setting; moreover, the study population should be homogenous about clinical characteristics.

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### Materials and Methods

We constructed tissue microarrays with paraffin-embedded specimens ( $n = 500$ ) from patients enrolled in a randomized clinical trial between 1984 and 1991. The trial included 564 patients who were premenopausal or aged <50 years and had been diagnosed with stage II invasive breast cancer. The aim of the original study was to compare

**Table 1.** Distribution of and relations between ER and PR subgroups

ER fraction	PR fraction				Unknown	Total
	0-10%	11-50%	51-75%	>75%		
0-10%	130	7	0	6	8	151
11-50%	7	15	8	18	4	52
51-75%	9	11	7	27	4	58
>75%	14	35	39	114	12	214
Unknown	3	2	2	3	15	25
Total	163	70	56	168	43	500

2 years of tamoxifen treatment (20-40 mg daily) with no adjuvant treatment. No stratification for tumor size or nodal status was made, and patients were included irrespective of hormone receptor status. The median follow-up time for patients without breast cancer events was 13.9 years, equal in both arms. Details of the study design have been reported elsewhere (19). The study has been approved by the ethical committees at Lund and Linköping Universities.

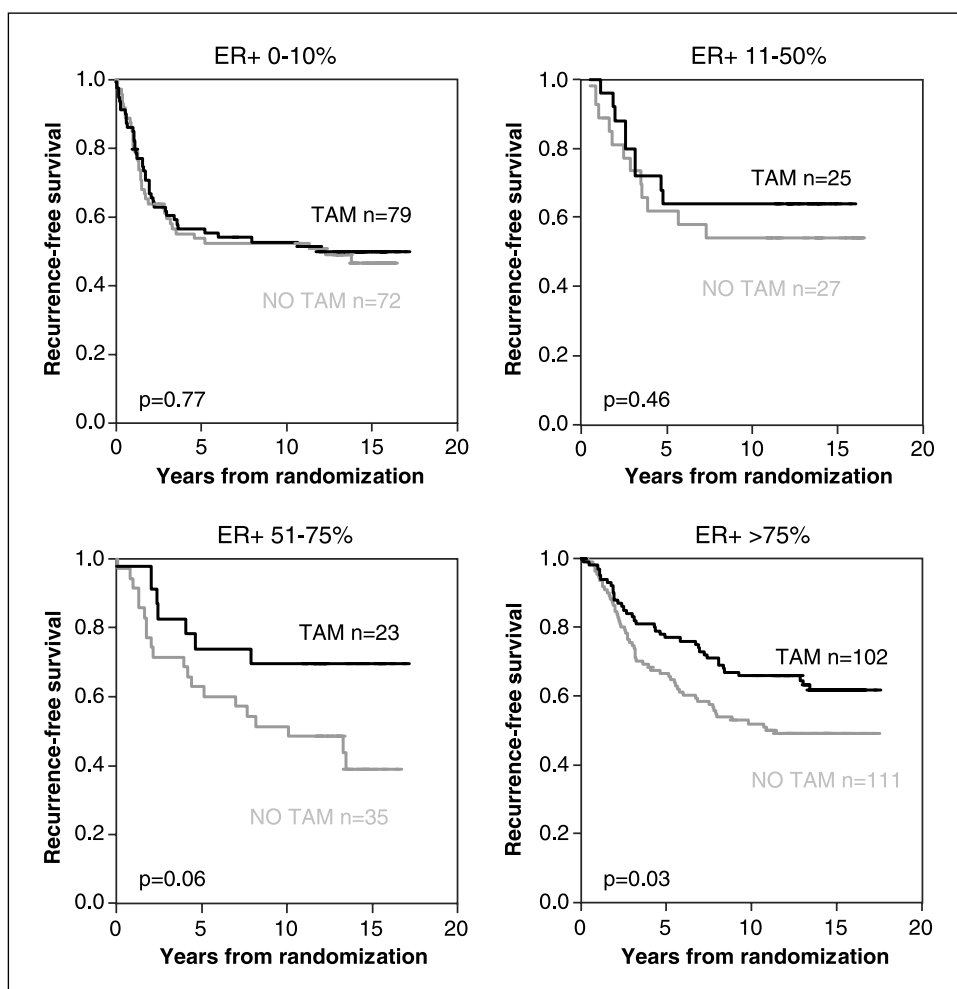
For this study, all H&E-stained slides were reevaluated and six 0.6-mm tissue cores were taken from representative invasive areas of the paraffin-embedded tumor material and mounted pairwise in triplicate recipient blocks using an automated arrayer (ATA-27, Beecher, Inc., Sun

Prairie, WI). Immunohistochemical staining of ER and PR was done on 4- $\mu$ m sections using the Ventana Benchmark system (Ventana Medical Systems, Inc., Tucson, AZ) with prediluted antibodies (anti-ER clone 6F11 and anti-PR clone 16). The fractions of positive tumor cell nuclei were subgrouped as 0 (0-10%), 1 (11-50%), 2 (51-75%), and 3 (76-100%). All evaluations were made by a pathologist blinded to patient information. Because assessment of the nuclear staining intensity is not part of the diagnostic routines in Sweden, this variable was not taken into account. However, a high nuclear fraction was generally associated with a strong nuclear staining intensity in the tumors investigated in this study.

In the first report of this trial (19), ER and PR status was evaluated either by cytosolic measurements ( $n = 453$ ) or immunohistochemistry ( $n = 88$ ). The immunohistochemical assessment of the hormone receptors was done on a manually constructed array containing two 0.6-mm tissue cores per case. For this study, ER status of all tumors was assessed in an additional set of automatically constructed arrays and PR status was evaluated and compared in triplicate, automatically constructed arrays as a validation of the tissue microarray technique. Tumor material was available from 500 of the original 564 patients enrolled in the clinical trial.

Recurrence-free and overall survival were estimated according to the Kaplan-Meier method, and the log-rank test was used to compare survival in different strata. Recurrence-free survival considered local, regional, distant recurrences, and breast cancer death but not contralateral breast cancer as primary events. Tamoxifen treatment has been found to reduce the risk of contralateral breast cancer in both premenopausal and postmenopausal patients (1, 21). A contralateral

**Fig. 1.** Recurrence-free survival according to ER levels comparing tamoxifen-treated patients (TAM) with untreated patients (NO TAM).



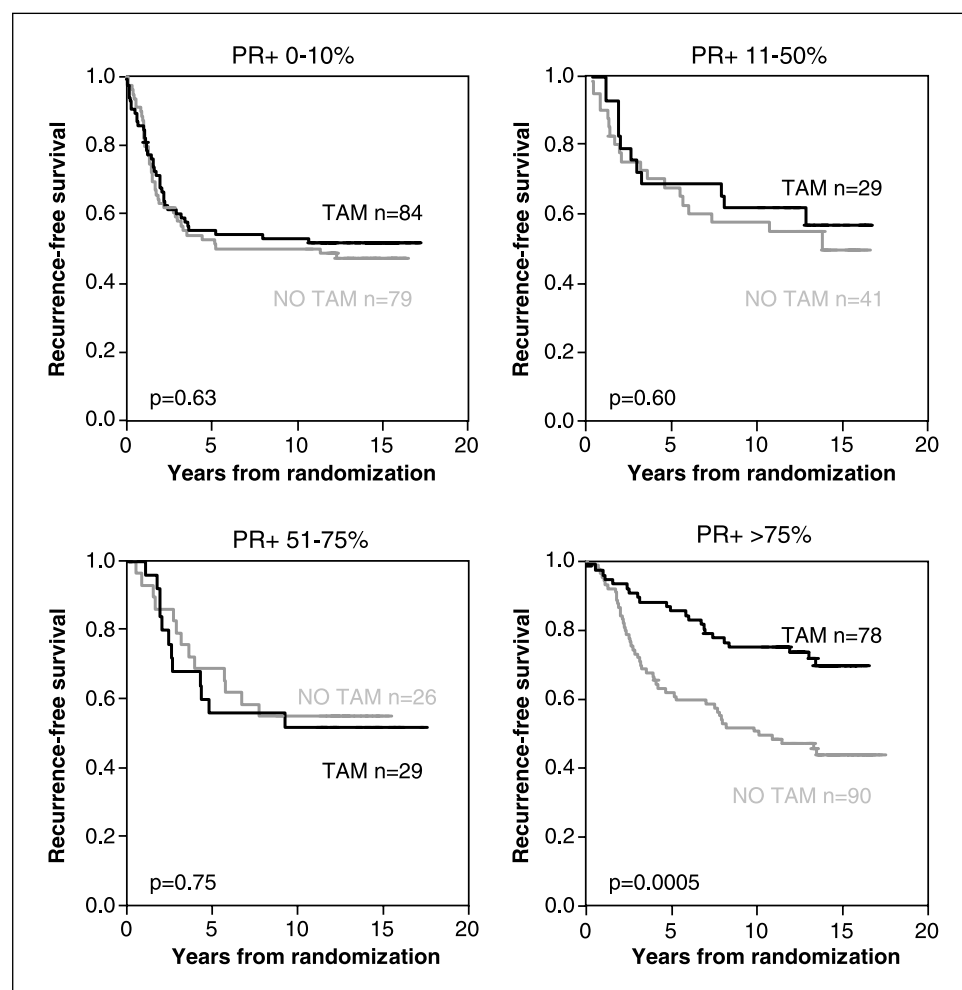


Fig. 2. Recurrence-free survival according to PR levels comparing tamoxifen-treated patients (TAM) with untreated patients (NO TAM).

event usually occurs >5 years after the original tumor, and it can be difficult to determine whether it should be regarded as a recurrence or as a new primary; therefore, this entity was not included in the analysis.

A Cox proportional hazards model was used for the estimation of the relative risk in univariate and multivariate analyses. The interaction between tamoxifen treatment and the investigated variables was further explored by a Cox model, including one of the four variables, respectively, a treatment variable, and an interaction variable. All statistical tests were two sided, and the calculations were done in Statistical Package for the Social Sciences version 11.0 (SPSS, Inc., Chicago, IL).

## Results

Immunohistochemical evaluation of ER status was possible in 474 of 500 (95%) cases, and of these, 323 (68%) cases were positive according to clinical standards (>10%). PR was evaluable in 456 (91%) cases with 293 (64%) cases having >10% positive cells, and 168 (37%) of these had >75% positive cells (Table 1). From the ER expression level of >10%, a gradually improving recurrence-free survival on tamoxifen treatment was observed, with higher levels of ER further improving patient outcome (Fig. 1). However, assessment of PR status in the ER-positive subgroup (>10%, reflecting the clinically used cutoff) revealed that PR levels were strongly predictive of tamoxifen response in that tumors with PR levels

<75% showed virtually no response compared with the control group, whereas PR levels >75% clearly predicted a significant treatment effect for recurrence-free [relative risk, 0.41 (0.24-0.69);  $P = 0.001$ ] as well as overall [relative risk, 0.46 (0.26-0.83);  $P = 0.009$ ] survival. Notably, these findings were consistent in all tumors irrespective of ER status (Fig. 2) and at different ER levels (Fig. 3). Statistical results were unchanged irrespective of the number of evaluable tissue cores in each tumor, further validating the tissue microarray technique. In a multivariate interaction analysis using a Cox regression model, a significant interaction between tamoxifen treatment and PR status dichotomized at 75% could be shown both for recurrence-free [relative risk, 0.48 (0.26-0.88);  $P = 0.018$ ] and overall [relative risk, 0.52 (0.28-0.99);  $P = 0.048$ ] survival. When adjusted for established prognostic factors, significance improved slightly (data not shown).

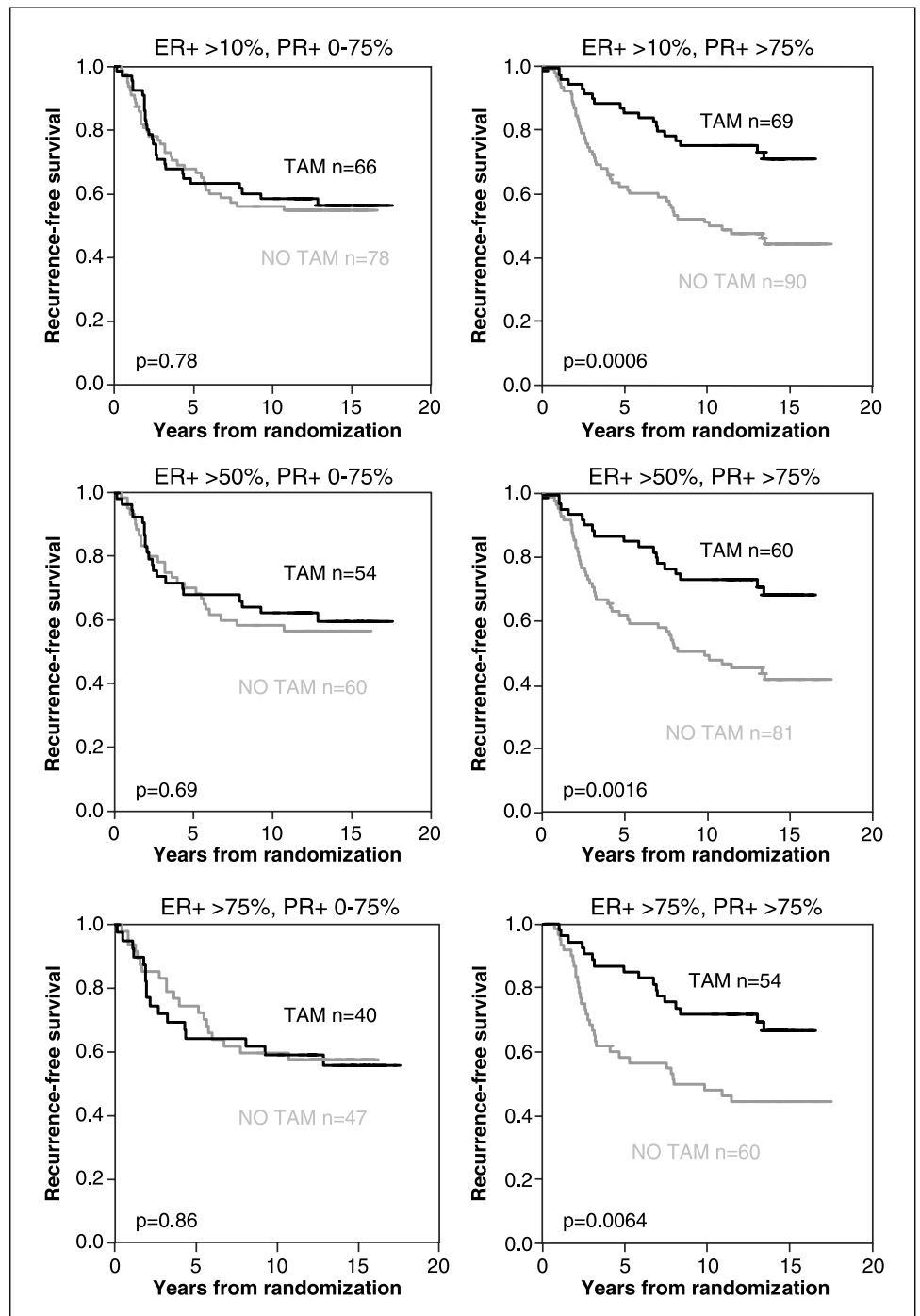
## Discussion

The findings from this randomized trial with long-term follow-up clearly show the indisputable value of the PR as a predictor of tamoxifen response. Irrespective of the cutoff chosen for the ER, neither recurrence-free nor overall survival was improved by tamoxifen for patients with tumors with a PR

content of <75% compared with untreated patients. In contrast, both recurrence-free and overall survival improved significantly on tamoxifen treatment in patients with tumors showing >75% PR-positive nuclei. The PR is located downstream of the ER, and the prevailing theory has long been that the amount of PR in a tumor potentially reflects a functioning ER pathway, thereby predicting the effect of endocrine treatment. However, recent studies show that ER<sup>+</sup>/PR<sup>-</sup> tumors may be resistant to tamoxifen but respond to aromatase inhibitors (20), suggesting a still functioning ER mechanism. Instead, tamoxifen resistance has been attributed to cross talk between ER and growth factor

signaling pathways that down-regulate PR while activating other ER functions (22).

It is important to note that our study has been carried out in premenopausal patients who are less suitable for treatment with aromatase inhibitors. In fact, tamoxifen is still the only efficient endocrine treatment available for these patients, as treatment with aromatase inhibitors demands ovarian suppression and this in turn leads to unwanted side effects. Tamoxifen treatment has side effects as well, and it is therefore of importance to identify the patients that do benefit from the treatment. PR is often analyzed in breast cancer tumors but rarely taken into account.



**Fig. 3.** Recurrence-free survival according to PR levels at various ER levels comparing tamoxifen-treated patients (*TAM*) with untreated patients (*NO TAM*).

Our results suggest that PR status indeed provides very useful predictive information. The purpose of the study was to refine the evaluation of hormone receptor status by applying a quantified rather than dichotomized assessment. This approach has the disadvantage of rendering smaller subgroups and, consequently, loss of statistical power. Therefore, our results need to be confirmed in additional studies. However, they are

promising in that a fractioned evaluation of immunohistochemical hormone receptor expression could easily be adopted and incorporated into clinical practice and clinical trials.

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