GYNECOLOGY

Impact of hormonal biomarkers on response to hormonal therapy in advanced and recurrent endometrial cancer

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BACKGROUND: Approximately 20% of women with endometrial cancer have advanced-stage disease or suffer from a recurrence. For these women, prognosis is poor, and palliative treatment options include hormonal therapy and chemotherapy. Lack of predictive biomarkers and suboptimal use of existing markers for response to hormonal therapy have resulted in overall limited efficacy.

OBJECTIVE: This study aimed to improve the efficacy of hormonal therapy by relating immunohistochemical expression of estrogen and progesterone receptors and estrogen receptor pathway activity scores to response to hormonal therapy.

STUDY DESIGN: Patients with advanced or recurrent endometrial cancer and available biopsies taken before the start of hormonal therapy were identified in 16 centers within the European Network for Individualized Treatment in Endometrial Cancer and the Dutch Gynecologic Oncology Group. Tumor tissue was analyzed for estrogen and progesterone receptor expressions and estrogen receptor pathway activity using a quantitative polymerase chain reaction—based messenger RNA model to measure the activity of estrogen receptor—related target genes in tumor RNA. The primary endpoint was response rate defined as complete and partial response using the Response Evaluation Criteria in Solid Tumors. The secondary endpoints were clinical benefit rate and progression-free survival.

RESULTS: Pretreatment biopsies with sufficient endometrial cancer tissue and complete response evaluation were available in 81 of 105 eligible cases. Here, 22 of 81 patients (27.2%) with a response had estrogen and progesterone receptor expressions of >50%, resulting in a response rate of 32.3% (95% confidence interval, 20.9—43.7) for an estrogen receptor expression of >50% and 50.0% (95% confidence interval, 35.2—64.8) for a progesterone receptor expression of >50%. Clinical benefit rate was 56.9% for an estrogen receptor expression of >50% (95% confidence interval, 44.9—68.9) and 75.0% (95% confidence interval, 62.2—87.8) for a progesterone receptor expression of >50%. The application of the estrogen receptor pathway test to cases with a progesterone receptor expression of >50% resulted in a response rate of 57.6% (95% confidence interval, 42.1—73.1). After 2 years of follow-up, 34.3% of cases (95% confidence interval, 20—48) with a progesterone receptor expression of >50% and 35.8% of cases (95% confidence interval, 20—52) with an estrogen receptor pathway activity score of >15 had not progressed.

CONCLUSION: The prediction of response to hormonal treatment in endometrial cancer improves substantially with a 50% cutoff level for progesterone receptor immunohistochemical expression and by applying a sequential test algorithm using progesterone receptor immunohistochemical expression and estrogen receptor pathway activity scores. However, results need to be validated in the prospective Prediction of Response to Hormonal Therapy in Advanced and Recurrent Endometrial Cancer (PROMOTE) study.

Key words: aromatase inhibitors, estrogen receptor pathway activity, progesterone receptor, progestin therapy

Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in Western countries, and its incidence is increasing.[1,2] In most patients, EC is diagnosed when the disease is confined to the uterus and the outcome is favorable.[3] However, 20% of patients present with advanced-stage disease or develop a recurrence.[4–6] For these patients, the prognosis is poor, and treatment options are limited. In the palliative setting, chemotherapy and hormonal therapy are the most frequently applied treatments. First-line chemotherapy has a response rate (RR) of approximately 60% but is associated with grade 3 to 4 toxicity in approximately 50% of patients.[7–9] Hormonal therapy has an RR of 20% to 40% in an unselected population, with serious side effects in <5% of patients.[10–13] Hormonal drugs, mainly in the form of progestins, tamoxifen, and aromatase inhibitors, are used to inhibit the proliferative effects of estrogen on tumor growth.[14] Theoretically, hormonal therapy is most effective when tumor growth is estrogen dependent. However, a good biomarker to identify estrogen-mediated tumor growth is currently lacking. Therefore, optimization of existing biomarkers and

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AJOG at a Glance

Why was this study conducted?

Chemotherapy and hormonal therapy are the most common treatment modalities for advanced and recurrent endometrial cancer. Hormonal therapy is an attractive treatment option because of limited toxicity; however, the response rate (RR) is only 20% to 40%. In this study, estrogen and progesterone receptor (PR) expressions and estrogen receptor pathway activity (ERPAS) in pretreatment biopsies were related to RR and progression-free survival.

Key findings

PR expression of >50% resulted in an RR of 50%; a combined PR expression of >50% and positive ERPAS yielded a response rate of 57.6%. Moreover, 34.3% of cases with a PR expression of >50% and 35.8% of women with a positive ERPAS had not progressed after 2 years of follow-up.

What does this add to what is known?

The prediction of response to hormonal therapy can be substantially improved with PR expression and ERPAS as determined in pretreatment biopsies.

identification of new biomarkers are essential to tailor hormonal treatment to patients based on the predicted response.[15−18]

Estrogen receptor alpha (ER) and progesterone receptor (PR), as evaluated with immunohistochemical expression (IHC), are currently used biomarkers for prognosis and response to hormonal therapy with superior outcome for ER or PR positive ECs compared to ER or PR negative ECs.[10,11,19,20] Contrary to breast cancer, there is no uniform cutoff value for ER and PR IHC expressions. In clinical practice, 10% of tumor-positive nuclei is a frequently used cutoff value for prognosis with consistent relations with disease-specific and disease-free survival. In the predictive setting, there is a substantial variety in applied cutoffs, but there is no study that has investigated an optimal cutoff value from a range of values.[10,21] In addition, predictive biomarker analyses in previous studies were mainly performed on archival tumor instead of pretreatment biopsies, despite the fact that recent biopsies are known to better reflect actual ER or PR IHC status.[22−24] Finally, the presence of ER or PR is not always coupled with an active intracellular ER signaling that reflects hormone-driven tumor growth. Therefore, an ER pathway activity test that indicates an active ER signaling pathway might improve the prediction of response to hormonal therapy in EC. In breast cancer, high ER pathway activity scores (ERPAS) were associated with favorable prognosis and response to endocrine therapy.[25,26] In EC, ERPAS was recently demonstrated to better predict the prognosis than ER IHC expression.[27] To date, no study on ER pathway activity as a predictive marker in EC has been performed. Therefore, we analyzed hormonal markers that could improve the prediction of response to hormonal therapy in EC. Here, we reported the predictive value of ER and PR IHC expressions and ERPAS in pretreatment biopsies of patients included in the retrospective part of the Prediction of Response to Hormonal Therapy in Advanced and Recurrent Endometrial Cancer (PROMOTE-R study).

Materials and Methods

Study design and patients

The PROMOTE-R study is a retrospective study in which women with advanced-stage or recurrent EC treated with any type of hormonal therapy were identified from 16 medical centers within the European Network for Individualized Treatment in Endometrial Cancer (ENITEC) and the Dutch Gynaecology Oncology Group (DGOG). All participating centers obtained approval from institutional review boards or national ethical committees and obtained patient consent, according to local regulations. The Radboudumc Institutional Review Board approval number was 2017-3803.

The presence of a tumor biopsy that was taken no longer than 4 months before the start of hormonal therapy was mandatory for inclusion in this study. Biopsies were taken from tumor locations that were available for follow-up: in advanced-stage disease, this was either the uterus or a metastatic site, whereas in recurrent EC, it was a metastatic location Furthermore, follow-up of at least 3 months after the start of hormonal treatment was required. The exclusion criteria were as follows: the application of intercurrent treatment between biopsy and start of hormonal treatment, the application of previous hormonal therapy, and sarcoma or stroma cell sarcoma histology.

Endpoints and assessments

The primary endpoint was RR, and the secondary endpoints were clinical benefit rate (CBR) and progression-free survival (PFS). The effect of therapy was evaluated at 3 to 6 months after the start of hormonal therapy and categorized as complete response (CR), partial response, stable disease (SD), or progressive disease (PD). RR was calculated as the proportion of cases with complete or partial response. The CBR was defined as the proportion of cases with complete or partial response, or SD. The PFS was defined as the interval between the start of hormonal therapy and confirmation of progression or end of follow-up. The outcome was evaluated radiologically or clinically using the Response Evaluation Criteria in Solid Tumors (version 1.1).[28] Histologic complete regression of the tumor in follow-up tissue specimens was also defined as response. Evaluation based on clinical symptoms was only accepted in case of vaginal vault recurrences with blood loss. In these cases, a change in vaginal bleeding was considered a reliable indication for response to hormonal treatment: disappearance of vaginal blood loss was defined a CR, no change in blood loss was deemed SD, and increase in vaginal bleeding was regarded

as PD. The interval and frequency of follow-up could not be standardized owing to the retrospective nature of the study. Patients were not followed beyond PD. The outcome was reviewed by 3 members of the research team (W.J.V.W., R.I.L., and J.M.A.P.) independently to prevent bias in outcome definition in this cohort. Inconsistencies were solved in a consensus meeting. Reviewers were blinded to biomarker scores.

Data collection

ENITEC centers were requested to perform a retrospective search in the hospital database to identify women treated with hormonal therapy for advanced and recurrent EC up to 2016. For centers within DGOG, identification of eligible patients was facilitated by a search in the Netherlands Cancer Registry to identify all women diagnosed with advanced-stage EC and a search within the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA) for all women with recurrent EC.[29]

Immunohistochemical staining and scoring

IHC expressions for ER and PR were performed on 4-μm tumor-containing sections from formalin-fixed paraffin-embedded (FFPE) tumor blocks as described before.[27] Additional details, including antibodies used, are reported in Supplemental Material 1. The percentage of tumor cells expressing nuclear IHC ER and PR was independently evaluated by 2 of the researchers (W.J.V.W. and J.B.) with experience in ER and PR scoring.[19] Scoring was performed blinded for clinical data. In case of disagreement, the final score was decided in a consensus meeting.

RNA isolation and estrogen receptor pathway test

The tissue for RNA isolation was obtained from FFPE blocks after the presence of tumor tissue was confirmed by histology. The tissue of interest was then micro- or macrodissected, and RNA was extracted using the miRNeasy FFPE kit (Qiagen, Hilden, Germany) according to the instructions of the manufacturer with an extra step to prevent DNA contamination. The complete protocol is described in full in Supplemental Material 1.

The ER pathway activity test (version ER-O3.0b; Molecular Pathway Diagnostics, Philips, Eindhoven, the Netherlands; www.philips.com/oncosignal), with a list of included ER target genes, has been described before.[27,30] For this quantitative polymerase chain reaction model, a subset of the target genes were used. For the ER pathway activity test, a Bayesian computational model was used to infer ERPAS from the mRNA expression levels of ER target genes measured in tissue samples. Computed ERPAS was presented on a normalized scale between 0 and 100; the actual range of the ER pathway activity was laid on this scale and varied per tissue type. More information is out23lined in Supplemental Material 1 and Supplemental Figure 1.

Statistical analysis

The association between clinicopathologic findings and response to hormonal treatment was analyzed using the Student *t* test for continuous variables and the Fisher exact test for categorical data. For ER and PR IHC expressions, the optimal cutoff value was selected on the basis of sensitivity, specificity, positive predictive value, and negative predictive value with RR as endpoint. The optimal cutoff value for ERPAS was explored with a receiver operating characteristic curve, also with an RR as endpoint. A separate analysis among progestin users was performed as progestins are considered first-line hormonal treatment with the highest efficacy.[31] The 95% confidence intervals (95% CIs) for RR and CBR were calculated using the binomial normal approximation interval. The univariable Cox regression analysis was performed to study the association between established prognostic factors, such as tumor grade, tumor histology, ER IHC expression (cutoff value of 50%), PR IHC expression (cutoff value of 50%), and ERPAS with PFS. All factors with a significant association in univariable analyses were included in multivariable Cox regression analysis. Finally, Kaplan-Meier analysis with log-rank test was performed to estimate the cumulative PFS according to ER IHC expression, PR IHC expression, and ERPAS groups as defined above. *P* values of $<.05$ were considered to indicate a significant difference. The Statistical Package for Social Sciences statistical software (version 25; SPSS IBM, New York, NY) was used to perform the statistical analyses.

Results

Patients

A total of 103 eligible patients was identified in the PROMOTE-R study. Among those patients, 2 had 2 tissue samples taken that were evaluated separately, resulting in the inclusion of 105 cases. A total of 9 cases were excluded because the response could not be reliably evaluated: 4 cases because the evaluation was performed with symptoms with nonvaginal vault recurrences, 2 cases because the response evaluation within 6 months was lacking, 1 case because of incomplete clinical information, 1 case because of early-stage EC, and 1 case because hormonal therapy was already started at the time of biopsy. An additional 15 cases were excluded because the biopsies did not contain EC tumor tissue (n=7) after all diagnostic procedures were performed or the amount of tumor tissue was insufficient for analyses (n=8). A Consolidated Standards of Reporting Trials diagram is shown in Supplemental Figure 2. From the 81 remaining cases, ER IHC expression was available for 78 cases, PR IHC expression for 79 cases, and ERPAS for 73 cases.

Clinicopathologic characteristics of included cases are shown in the Table. The median age was 71.5 years, and the median body mass index was 30.1 kg/m^2. Hormonal therapy included progestin therapy in 79.0% (n=64), tamoxifen in 11.1% (n=9), and aromatase inhibitors in 9.9% (n=8) of cases. None of the patients used combinations of hormonal therapy. CR was observed in 8 cases (9.9%) and partial response occurred in 14 cases (17.3%), resulting in a RR of 27.2% (n=22). SD occurred in 18 cases (23.5%) and PD in 40 included

TABLE
Clinicopathological characteristics of included cases

Characteristic	Total number of cases (N=81)	Response (n=22 [27.2%])	No response (n=59 [72.8%])	*P* value
Age (y), SD	71.5 (62–81)	71.5 (60–82)	71.6 (62–81)	.97
BMI (kg/m^2), SD	30.1 (23–37)	28.3 (21–35)	30.9 (23–38)	.21
Grade[a]				
Grade 1–2	63	21 (33.3)	42 (66.7)	
Grade 3 EEC	9	0 (0)	9 (100.0)	
NEEC	4	0 (0)	4 (100.0)	.01[b]
Previous therapy				
Radiotherapy	32	10 (31.3)	22 (68.9)	.61[c]
Chemotherapy	5	3 (60.0)	2 (40.0)	.12[d]
Drug type				
Progestin	64	21 (32.8)	43 (67.2)	
Tamoxifen	9	0 (0)	9 (100.0)	
AI	8	1 (12.5)	7 (87.5)	.03[e]
Tumor type				
Advanced stage	30	6 (20.0)	24 (80.0)	
Recurrence	51	16 (31.4)	35 (68.6)	.31
Response evaluation				
History	6	1 (16.7)	5 (83.3)	
Examination	75	21 (28.0)	54 (72.0)	1.00
Clinical	14	6 (42.9)	8 (57.1)	
Radiologic	60	14 (23.3)	46 (76.7)	
Histologic	1	1 (100.0)	0 (0)	
Progressive disease				
Yes	69	10 (14.5)	59 (85.5)	
No	12	12 (100.0)	0 (0)	<.001

AI, aromatase inhibitor; *BMI*, body mass index; *EEC*, endometrioid-type endometrial cancer; *MPA*, medroxyprogesterone acetate; *SD*, standard deviation.

[a] Available for n=79; [b] Analysis for grade 1 and 2 EEC vs grade 3 EEC and NEEC; [c] Analysis for radiotherapy, yes or no; [c] Analysis for radiotherapy, yes or no; [d] Analysis for chemotherapy, yes or no; [e] Analysis for progestin vs tamoxifen or AI therapy.

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cases (49.4%). All cases with a response had grade 1 to 2 endometrioid-type endometrial cancer (EEC) histology. None of the tamoxifen users showed a response, whereas 1 case treated with an aromatase inhibitor had a response. For the complete cohort, the median follow-up time was 7.2 months (range, 2–83 months). Among 22 responders, the median follow-up time was 26.2 months (range, 4–83 months). In our study, 12 of 22 responders (45%) developed PD.

Response rate

The measured ER and PR IHC expressions and ERPAS among responders and nonresponders are shown in Figure 1. All patients with a response had an ER and PR IHC expressions of >50%. The application of the conventional 10% cutoff value resulted in an RR of 28.8% (95% CI, 18.4–39.2) for ER IHC expression and 37.3% (95% CI, 25.0–49.6) for PR IHC expression. The 1% cutoff yielded lower RRs than the 10% cutoff value (Supplemental Table 1). A cutoff value of 50% led to an RR of 32.3% (95% CI, 20.9–43.7) for ER IHC expression and 50.0% (95% CI, 35.2–64.8) for PR IHC expression. The analysis of higher cutoff values resulted in a higher RR, but the application of these cutoff values also resulted in loss of sensitivity. To maintain a sensitivity of 100%, subsequent tests were performed with the 50% cutoff value for ER and PR IHC expressions. For ERPAS, the optimal cutoff was defined using an ROC curve (Supplemental Figure 3). A cutoff of 15 was selected because of a sensitivity of 100% and a specificity of 70.4%. The RR of ERPAS of >15 was 54.3% (95% CI, 37.8–70.8).

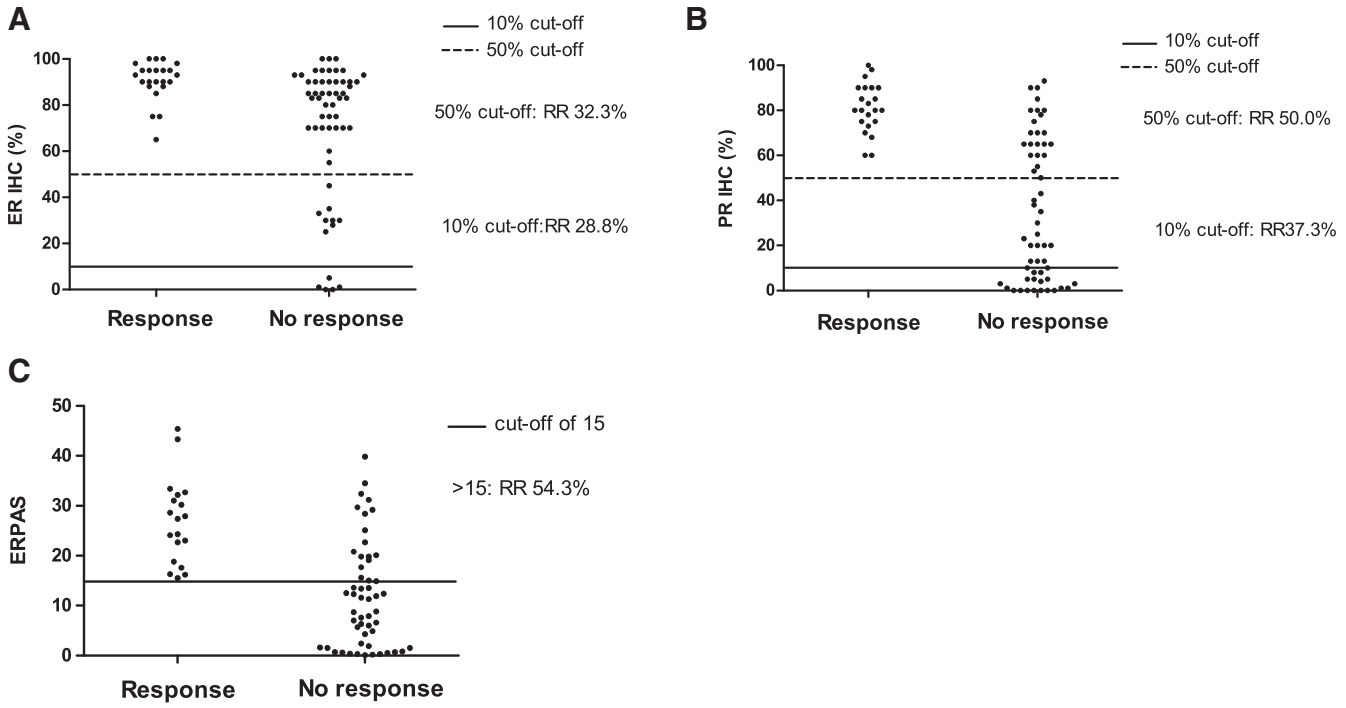
Sequential testing

The addition of ERPAS to ER and PR IHC expressions was explored in sequential test algorithms. The addition of ERPAS to cases with an ER IHC expression of >50% resulted in a change in RR from 32.3% (95% CI, 20.9–43.7) in the ER IHC expression of >50% group to 54.5% (95% CI, 41.7–67.3) in the ER IHC of >50% and ERPAS of >15 groups (Figure 2, A). The 13 cases (16.7% of included cases) with an ER IHC expression of ≤50% could be spared from ERPAS testing without impact on sensitivity. The application of ERPAS to cases with a PR IHC expression of >50%, resulted in an RR of 57.6% (95% CI, 42.1–73.1) in the PR IHC expression of >50% group and ERPAS of >15 groups. In this scenario, the 35 cases (44.3% of included cases) with a PR IHC expression of ≤50% would not require ERPAS testing (Figure 2, B).

Clinical benefit rate

The CBR, including cases with complete or partial response or stable disease, was 49.4% (n=40; 95% CI, 38.5–60.3). Cases with complete or partial response had significantly higher PR IHC expression and ERPAS than those with SD (Supplemental Figure 4). The application of the 10% cutoff value resulted in a CBR of 53.4% (95% CI, 42.0–64.8) for ER IHC expression and 62.7% (95% CI, 50.4–75.0) for PR IHC expression,

FIGURE 1
Hormonal biomarkers in relation to response to hormonal treatment



A, Relation of ER IHC to response. **B,** Relation of PR IHC to response. **C,** Relation of ERPAS to response. Response is defined as complete and partial responses. Nonresponse includes stable disease and progressive disease.

ER, estrogen receptor; *ERPAS*, ER pathway activity score; *IHC*, immunohistochemistry; *PR*, progesterone receptor.

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whereas a 50% cutoff value yielded a CBR of 56.9% (95% CI, 44.9−68.9) for ER IHC expression and 75.0% (95% CI, 62.2−87.8) for PR IHC expression (Figure 3). In cases with ER or PR IHC expression of ≤10%, the CBR was 0% and 15% (95% CI, 0−31), respectively. An ER IHC expression of 11% to 50% resulted in a CBR of 25% (95% CI, 5.0−55.0), and a PR IHC expression of 11% to 50% yielded a CBR of 26.7% (95% CI, 4.3−49.1). ERPAS of >15 was associated with a CBR in 74.3% of cases (95% CI, 60.5−88.8), and an ERPAS of ≤15 was associated with a CBR in 28.9% of cases (95% CI, 14.5−43.3).

Progestin treatment

As progestins were the most active hormonal drugs in this cohort, a separate analysis was performed for patients treated with progestins. For an ER IHC expression of >50%, the RR was 37.7% (95% CI, 24.7−50.7), and for PR IHC expression of >50%, the RR was 56.8% (95% CI, 40.8−72.8) (Figure 4). ERPAS >15 yielded a RR of 62.1% (95% CI, 44.4−79.8) in progestin users.

Progression-free survival

In the total cohort, 69 cases (85.2%) developed PD. In univariable regression analysis, grade 1 to 2, EEC histology, ER and PR IHC expressions of >50%, and ERPAS of >15 were significantly associated with a longer PFS (Figure 5, A). In multivariable regression analysis, including grade, histology, ER and PR IHC expressions, and ERPAS, an ERPAS of >15 was the sole marker that remained significantly associated with PFS (hazard ratio [HR], 4.525; 95% CI, 1.85−11.07; *P*=.001) (Figure 5, B). A separate multivariable regression analysis without ERPAS showed that PR IHC expression was the only variable with significant association with PFS (HR, 2.964; 95% CI, 1.58−5.58; *P*=.001; data not shown). The estimation of PFS with Kaplan-Meier analysis according to ER IHC expression, PR IHC expression, and ERPAS is shown in Figure 5, C−E. After 2 years of follow-up, 34.3% of cases (95% CI, 20−48) with a PR IHC expression of >50% and 35.8% of cases (95% CI, 20−52) with an ERPAS of >15 had not progressed.

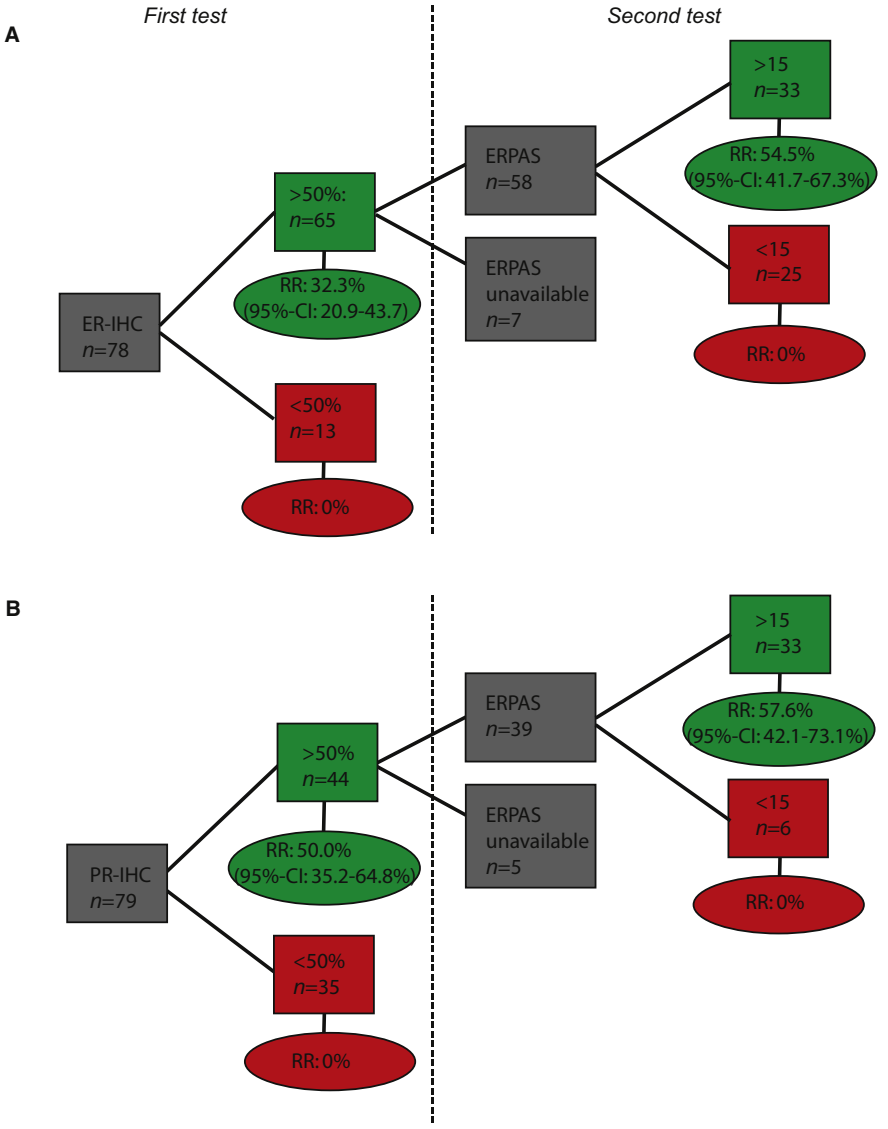
Correlation between biomarkers

The highest correlation was observed between PR IHC expression and ERPAS (Spearman rho, 0.786) (Supplemental Table 2). The correlation between ER IHC expression and ERPAS was 0.572 (Spearman rho).

Comment

Here, we evaluated different hormonal biomarkers in relation to response to hormonal therapy. The results indicated that PR IHC expression and ERPAS are the most important predictive

FIGURE 2
Sequential test algorithms of hormonal biomarkers for response to hormonal treatment



A, Sequential algorithm of ER IHC (first test) and ERPAS (second test). **B,** Sequential algorithm of PR IHC and ERPAS.

ER, estrogen receptor; *ERPAS*, estrogen receptor pathway activity score; *IHC*, immunohistochemistry; *PR*, progesterone receptor.

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biomarkers. The application of the 50% cutoff value for PR IHC expression resulted in an RR of 50.0% (95% CI, 35.2−64.8) and a CBR of 75.0% (95% CI, 62.2−87.8). Sequential testing of PR IHC expression with ERPAS identified a subset of patients with a response in 57.6% of cases.

PR IHC expression was found to have stronger associations with response to hormonal therapy than ER IHC expression. This was supported by results of the randomized trial by Thigpen et al[10] in which the PR IHC expression showed a stronger association with PFS than ER IHC expression among 299 women with advanced or recurrent EC treated with oral medroxyprogesterone acetate (MPA). Similarly, in a recent trial by Soliman et al,[32] 44 patients with advanced and recurrent EC were treated with everolimus, letrozole, and metformin. PR IHC expression was significantly associated with CBR, whereas ER IHC expression was not.

The ERPAS test has not been previously studied in relation to response to hormonal therapy in EC. However, in line with our study, ERPAS was shown to be significantly associated with response to neoadjuvant endocrine treatment in breast cancer.[25] Interestingly, our study showed that the correlation between ER pathway activity and PR IHC expression was higher than the correlation between ER pathway activity and ER IHC expression. This indicated that PR IHC expression might be a more accurate marker for downstream estrogen signaling activity than ER IHC expression.

Other potential biomarkers included tumor grade and histology, although these markers showed limited positive predictive value in our cohort. As the group of grade 3 tumors was small, the analysis on the predictive role of ERPAS and PR IHC expression was not feasible. A larger cohort with high-grade ECs is required to determine if the application of hormonal treatment based on ERPAS and PR IHC expression is justified in this group.

Mismatch repair deficiency markers are also interesting markers that have been related to poor response in young patients with stage 1 EC who wish to preserve fertility.[33] It is currently not clear if these markers are also relevant in advanced and recurrent ECs.

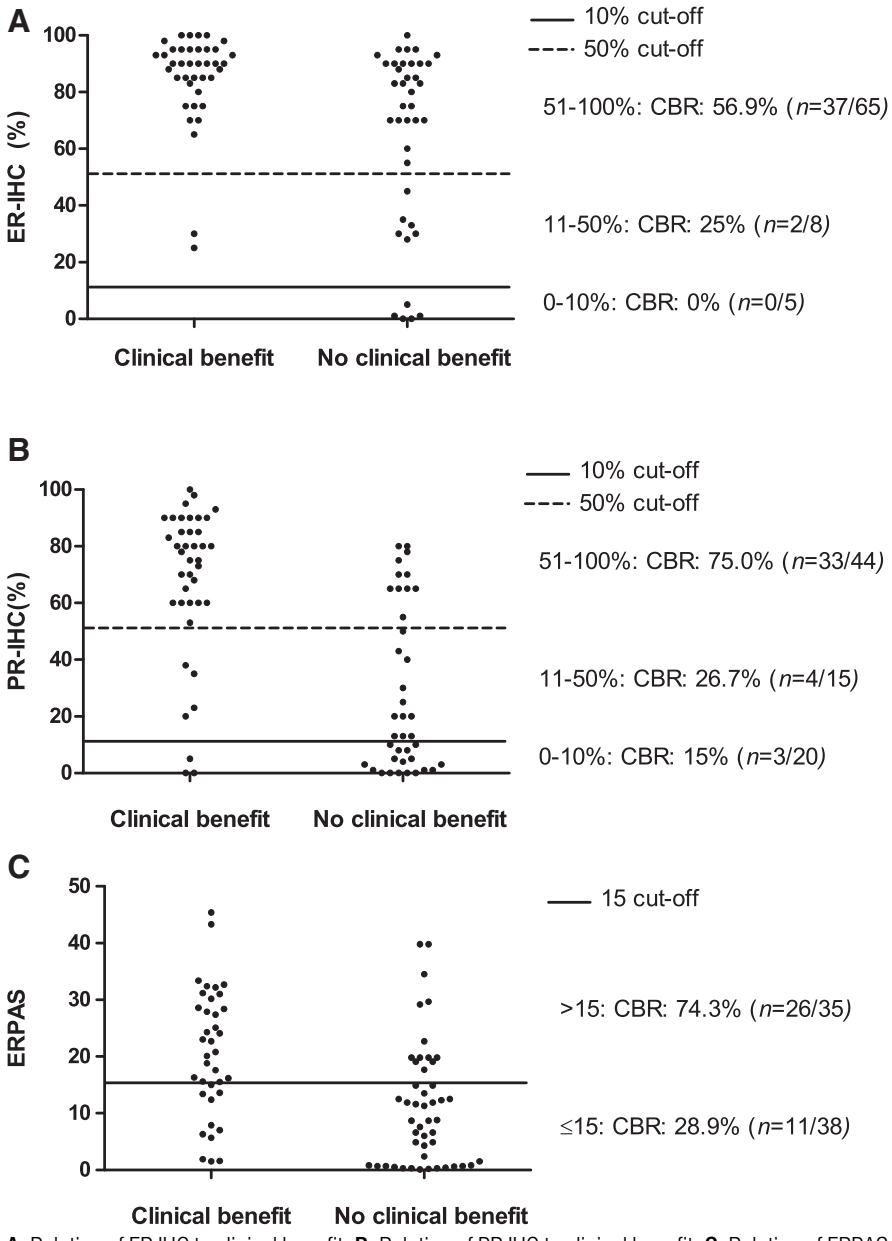
The highest RR in our study was observed for cases with a PR IHC expression of >50% and an ERPAS of >15. Sequential testing with a PR IHC expression of >50% and an ERPAS of >15 could also limit the number of cases that require ERPAS testing without affecting sensitivity, indicating that the sequential test algorithm could assist in reducing the costs for ERPAS testing. Both the additional benefit and cost-effectiveness of ERPAS need to be validated in a prospective study.

The endpoint CBR was selected to include all patients with a benefit from

hormonal therapy as prolonged SD can be considered a favorable outcome in a palliative setting. In our cohort, there was a selection of cases with an ERPAS of ≤15 or a PR IHC expression of ≤50% that showed SD, questioning the predictive value of ERPAS and PR IHC expression for SD. However, the evaluation for SD at 3 to 6 months after the start of therapy might be too short to observe tumor growth especially in grade 1 to 2 EECs with indolent tumor growth. Prolonged SD of more than 6 or 12 months might be a better measure for the effectiveness of treatment. Indeed, PR IHC expression and ERPAS were significantly higher for cases with an SD of >12 months than cases with an SD of ≤12 months (data not shown). Progestins were the most active type of drugs in this cohort, and the RRs were highest for progestin-treated patients with a PR IHC expression of > 50% and positive ERPAS. Mechanistically, progestins counteract estrogen-induced proliferation through activation of PR and modulation of ER action in both the menstrual cycle and breast and endometrial neoplasias.[34,35] The response to tamoxifen and aromatase inhibitors was low in line with studies that show lower RR compared with progestins.[31,36,37] However, in this study, some centers exclusively used tamoxifen and aromatase inhibitors in PR-negative disease. Aside from its predictive relevance, PR IHC expression is also a strong prognostic marker potentially impacting response to hormonal therapy and outcome in cases with PR-negative disease.[19,20] In our study, 50% of tamoxifen and 25% of aromatase inhibitor users had a PR IHC expression of ≤10%. Therefore, a larger cohort of patients with nonprogestin hormonal drugs is necessary to validate the role of PR IHC expression and ERPAS.

Here, the overall RR for hormonal therapy was comparable with other studies in similar populations. For example, in the study by Thigpen et al,[10] 25% of patients treated with MPA 200 mg/day responded. Similarly, Lentz et al[11] reported an RR of 24% for treatment with megestrol acetate.

FIGURE 3
Hormonal biomarkers in relation to clinical benefit rate for hormonal therapy



A, Relation of ER IHC to clinical benefit. **B,** Relation of PR IHC to clinical benefit. **C,** Relation of ERPAS to clinical benefit. Clinical benefit is defined as complete response, partial response, and stable disease. Nonresponse includes progressive disease.

ER, estrogen receptor; *ERPAS*, estrogen receptor pathway activity score; *IHC*, immunohistochemistry; *PR*, progesterone receptor.

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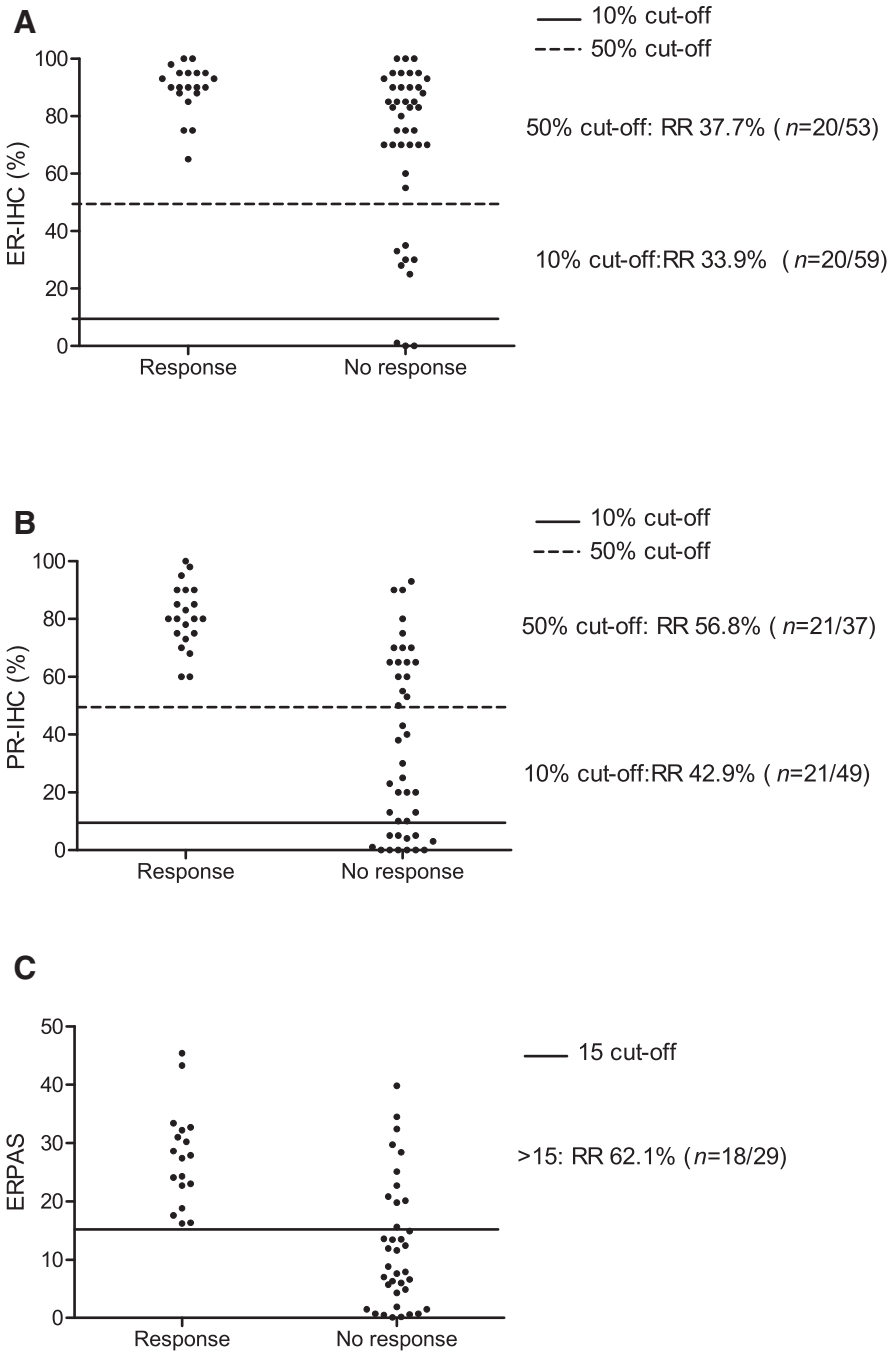
Furthermore, a subset of patients with higher RR could be identified on the basis of PR IHC expression and ERPAS. Relating the response to tissue biomarkers was facilitated by limiting inclusions to patients with available pretreatment biopsies as opposed to analyzing earlier taken tumor samples.[10,13,38] Performing biomarker analysis on pretreatment biopsies instead of primary tumors is relevant because PR-positive primary tumors have shown to become PR negative in up to 50% of metastasis.[22–24] Therefore, archival

FIGURE 4
Hormonal biomarkers in relation to response rate for progestin therapy



A, Relation of ER IHC to response. **B,** Relation of PR IHC to response. **C,** Relation of ERPAS to response. Response is defined as complete and partial responses. Nonresponse includes stable disease and progressive disease.

ER, estrogen receptor; *ERPAS*, estrogen receptor pathway activity score; *IHC*, immunohistochemistry; *PR*, progesterone receptor.

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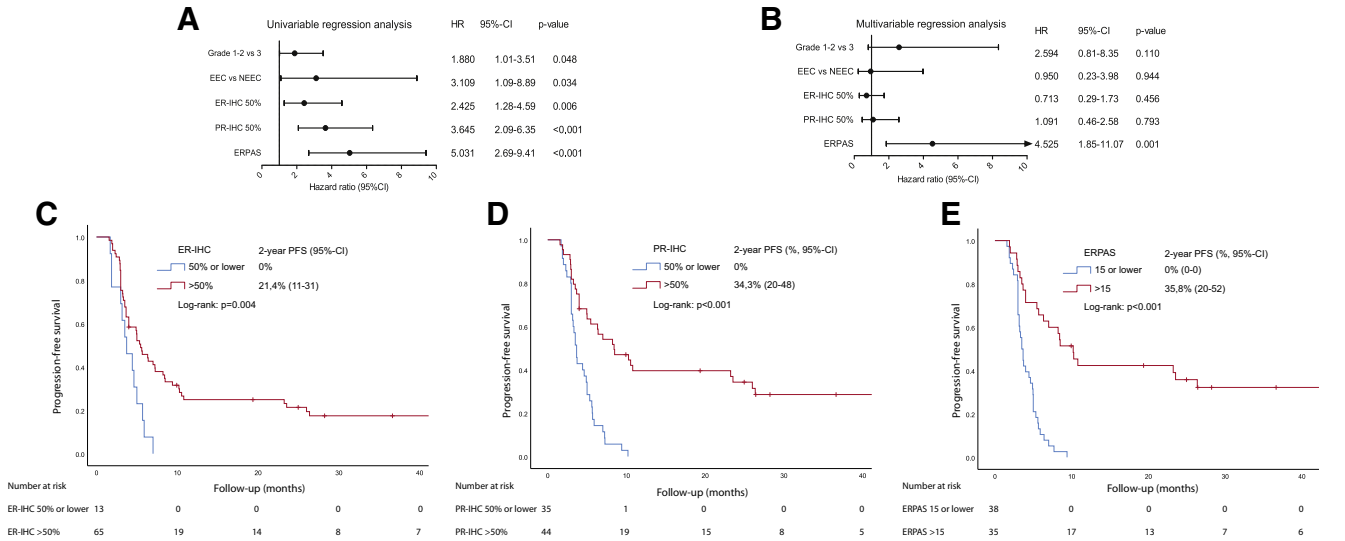
tumor tissue does not adequately reflect the PR IHC status of the metastasis that is treated with hormonal therapy. Another factor that has contributed to the identification of a subgroup with a higher RR is the identification of ER and PR IHC cutoff values with the best association with outcome. Earlier studies have used cutoff values of 10% of positive tumor nuclei or cutoff values on combined staining intensity indices.[21,32,39] A cutoff of 1%, as is currently advised in breast cancer, was also inferior to the 50% cutoff value.[40] Another potential advantage of the 50% cutoff value is that it is in use for other predictive biomarkers and it is easy to apply when scoring IHC slides.[41] The reported RR and CBR indicated that hormonal therapy has a place in the treatment of advanced and recurrent EC if applied to patients with a PR IHC expression of >50% and/or positive ERPAS.

Strengths and Limitations

The strengths of this retrospective study include the selection of cases with a pretreatment biopsy, the evaluation for outcome by 3 independent reviewers, and the exploration of multiple cutoff levels in relation to outcome. However, there are also limitations to be addressed. First, this cohort was a highly selected population in which hormonal therapy was considered a viable treatment option by the treating physician. The mandatory availability of a pretreatment biopsy and the fact that 25 of 81 inclusions (30.8%) had grade 1 to 2 stage I EC limited the application of radio- and chemotherapy in this cohort. It is unclear whether the same RRs can be obtained in more heavily pretreated patient groups. The relation between response to hormonal therapy and previous radio- or chemotherapy needs to be explored to strengthen the position of hormonal therapy in advanced and recurrent EC. Second, 6 cases (7.4%) were evaluated on the basis of symptoms because more objective methods of evaluation were unavailable. To limit inaccuracy, evaluation based on complaints was only accepted for vaginal vault recurrences in which vaginal blood loss was regarded as a reliable method of evaluation. The exclusion of these 6 cases did not alter the study results. Third, no standardized follow-up regimen for PFS was possible because of the retrospective

FIGURE 5
Predictive biomarkers in relation to PFS



Univariable (**A**) and multivariable (**B**) Cox regression analysis for the association of predictive markers with PFS (**C**—**E**): Kaplan-Meier analysis with log-rank test for the (**C**) association of ER IHC, (**D**) PR IHC, and (**E**) ERPAS with progression-free survival. A *P* value of $<.05$ indicates significant association.

CI, confidence interval; *EEC*, endometrioid-type endometrial cancer; *ER*, estrogen receptor; *ERPAS*, estrogen receptor pathway activity score; *HR*, hazard ratio; *IHC*, immunohistochemical analysis; *NEEC*, nonendometrioid endometrial cancer; *PFS*, progression-free survival; *PR*, progesterone receptor.

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nature of this study. Therefore, validation of PFS results is indicated. The prospective PROMOTE study will include regular computed tomography scans or clinical evaluations to facilitate analysis for PFS (NCT03621904 on http://www.clinicaltrials.gov/). Forms and dosages of hormonal therapy could have influenced the outcome in our cohort. Currently, there is no consensus on the optimal dosage for any type of hormonal drug, although 1000 mg MPA was shown to be less effective compared with a lower dose of 200 mg MPA/day.[10] In this cohort, common dosages were used, and no impact of dosage on outcome was observed (data not shown).

Conclusion

We have shown that PR IHC expression and ER pathway activity have the highest predictive value for response and PFS for hormonal therapy in advanced and recurrent EC. For ER and PR IHC expression, a new cutoff value of 50% of positive tumor nuclei was proposed on the basis of relation with response. A sequential test algorithm with PR IHC expression and ERPAS identified a subset of patients with the most favorable outcome. Both the new cutoff value for PR IHC expression and the additional value of ERPAS will be validated in the prospective PROMOTE study. ■

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Philips Research has performed the ER pathway analysis free of charge. Philips Research was not granted access to patient details and did not have a role in the interpretation or representation of results. The analyses were also performed in Netherlands.

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Supplemental Material 1

Hormonal biomarkers can improve the prediction of response to hormonal therapy in advanced and recurrent endometrial cancer: results of the Prediction of Response to Hormonal Therapy in Advanced and Recurrent Endometrial Cancer.

Immunohistochemical staining protocol

Here, 4-μm tumor-containing sections were mounted on Superfrost slides. After antigen retrieval with EnVision FLEX target retrieval solution HIGH (Dako, Agilent, Santa Clara, CA) and blocking of endogenous peroxidase with EnVision FLEX Peroxidase-Blocking Reagent (Dako, Agilent, Santa Clara, CA), slides were incubated with either estrogen receptor (ER) alpha antibody (SP1 RM-9101-S, Thermo Scientific Immunologic, Waltman, MA) diluted 1:1600 in normal antibody diluent (Immunologic BV, Duiven, the Netherlands), progesterone receptor antibody (PgR636 M356901, Dako, Agilent, Santa Clara, CA) diluted 1:500 in normal antibody diluent or androgen receptor antibody (EP120 200R-26, Cell Marque Sanbio, Uden, the Netherlands) diluted 1:200 in normal antibody diluent. The slides were subsequently incubated with EnVision FLEX/HRP (Dako, Agilent, Santa Clara, CA), and then visualized with EnVision FLEX DAB+ substrate chromagen (Dako, Agilent, Santa Clara, CA). Finally, the slides were counterstained with hematoxylin, dehydrated, and mounted. For internal control, the liver and breast (ER immunohistochemical), breast and tonsil (progesterone receptor immunohistochemical), and kidney, lung, prostate and ovarian tissue (androgen receptor immunohistochemical) were used.

RNA isolation protocol

Tissue of interest was marcodissected from 4 tumor-containing slides of 10 μm or microdissected from 10 slides of 4 μm in case of scarce tumor material. The tumor tissue was then transferred into 1.5-mL microcentrifuge tubes. RNA was extracted using the miRNeasy FFPE Kit (Qiagen, Hilden, Germany) with an optimized protocol. First, the tissue was incubated for 15 minutes at 60°C and 15 minutes at 80°C at 1000 rpm with 240 μL Proteinase K digest buffer and 50 μL Proteinase K, after which 500 μL buffer red blood cell was added. The sample was transferred into a gDNA Eliminator Spin Column (Qiagen, Hilden, Germany) and centrifuged for 30 seconds at 10,000 rpm. The flow-through was mixed with 1200 μL 100% ethanol, and 700 μL of the sample was transferred to a new RNeasy MinElute spin column and centrifuged for 15 seconds at 10,000 rpm; the flow-through was discarded. This step was repeated until the entire sample had passed through, after which 500 μL buffer was added for washing of membrane-bound RNA, and the sample was centrifuged for 15 seconds at 10,000 rpm. Another 500 μL buffer was added, and the sample was centrifuged for 2 minutes at 10,000 rpm. The spin column was then placed in a new 2-mL microcentrifuge tube and centrifuged for 5 minutes with an open lid at full speed. After the flow-through was discarded, the spin column was placed in a new 1.5-mL microcentrifuge tube, and 30 μL RNase-free water was put directly onto the membrane of the spin column and centrifuged for 1 minute at full speed with a closed lid. This step was repeated to generate higher yield and total volume.

Estrogen receptor pathway activity score test

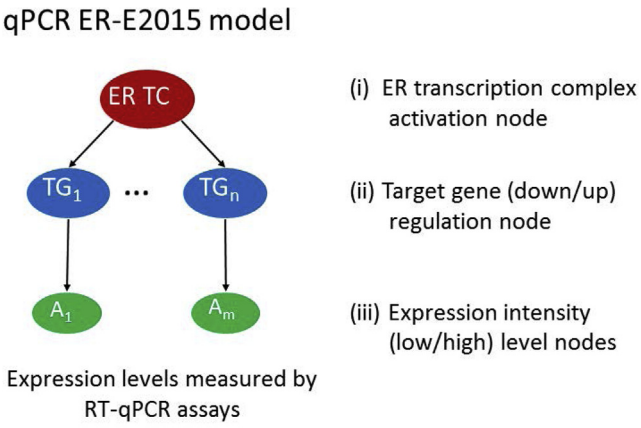
A Bayesian network representing the estrogen receptor (ER) pathway transcriptional program described how target gene regulation depends on ER transcription complex activity and how expression level intensities in turn depend on regulation of the respective target genes (Supplemental Figure 1). The network consisted of 3 types of nodes: (1) an ER transcription complex activation node; (2) target gene regulation nodes, with states "down" and "up"; and (3) expression intensity nodes, with states "low" and "high, each corresponding to an ER target gene. The quantitative polymerase chain reaction (qPCR)-based mRNA model was developed by Philips (qPCR ER-E2015 model, Philips Electronics, Eindhoven, the Netherlands; http://www.philips.com/oncosignal). The calibration of this model in breast cancer and EC has been described in detail before.[1,2]

Pathway scores were normalized such that the resulting values lay between 0 and 100, where 0 corresponds to the lowest odds for the pathway to be active and 100 corresponds to the maximum odds for pathway activity that the model can infer. All samples were analyzed in a blinded manner.

Supplemental References

1. van Weelden WJ, van der Putten LJM, Inda MA, et al. Oestrogen receptor pathway activity is associated with outcome in endometrial cancer. Br J Cancer 2020;123:785–92.
2. Inda MA, Blok EJ, Kuppen PJK, et al. Estrogen receptor pathway activity score to predict clinical response or resistance to neoadjuvant endocrine therapy in primary breast cancer. Mol Cancer Ther 2020;19:680–9.

SUPPLEMENTAL FIGURE 1
Schematic representation of qPCR model



Schematic representation of qPCR model

ER, estrogen receptor; *qPCR*, quantitative polymerase chain reaction; *RT-PCR*, reverse transcription-polymerase chain reaction.

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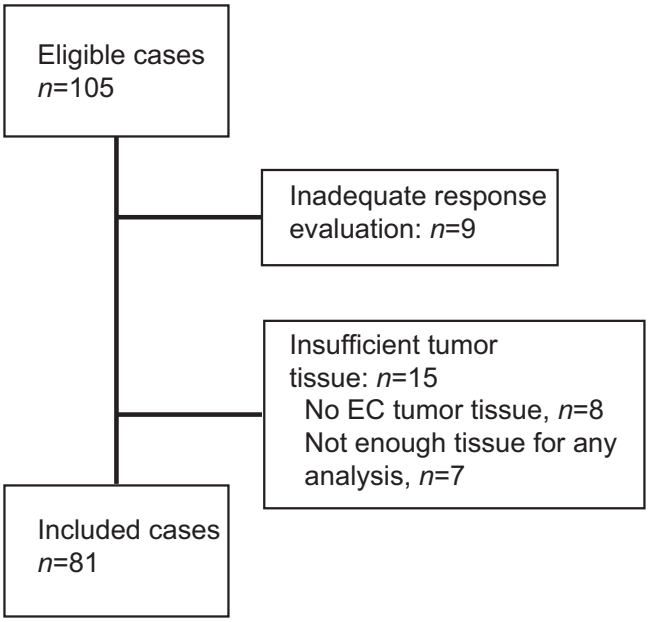
SUPPLEMENTAL FIGURE 2
CONSORT flow diagram



CONSORT, Consolidated Standards of Reporting Trials; *EC*, endometrial cancer.

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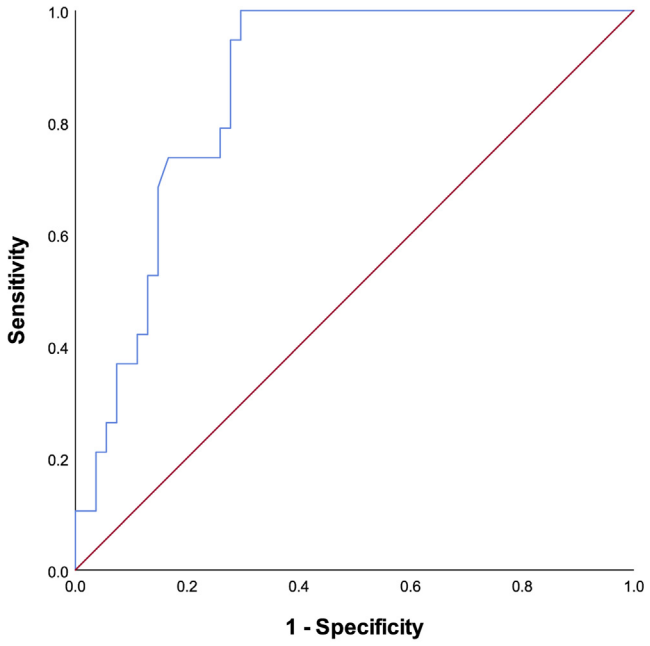
SUPPLEMENTAL FIGURE 3

Estrogen receptor pathway activity in relation to response to hormonal treatment



Receiver operating characteristic curve describing the sensitivity and specificity to predict the response to hormonal treatment. The area under the curve was 0.861. A cutoff value of 15 was used for the subsequent analyses as this value has a sensitivity of 1.00 and a 1-specificity of 0.296 in this cohort.

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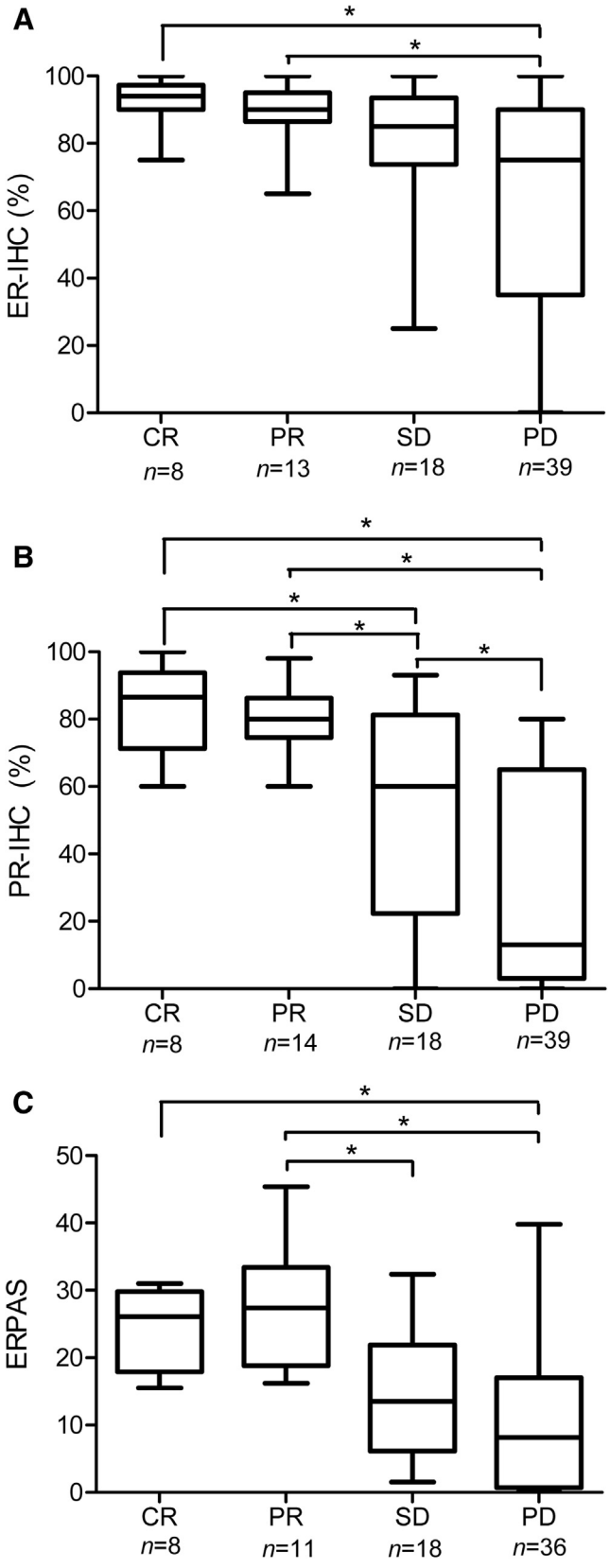
SUPPLEMENTAL FIGURE 4
Relation of response with hormonal biomarkers



A, ER IHC; **B,** PR IHC; **C,** ERPAS. The *asterisk* indicates significant difference at $P=.05$ in ANOVA with the Tukey HSD posthoc test.

ANOVA, analysis of variance; *CR*, complete response; *ER-IHC*, estrogen receptor expression with immunohistochemical analysis; *ERPAS*, estrogen receptor pathway activity scores; *IHC*, immunohistochemical analysis; *PD*, progressive disease; *PR*, partial response; PR-IHC, progesterone receptor with immunohistochemical analysis; *SD*, stable disease.

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SUPPLEMENTAL TABLE 1
Test characteristics of included biomarkers in relation to response to hormonal treatment

Biomarker	Cutoff (%)	n	TP (n)	FN (n)	TN (n)	FP (n)	Sens (%)	Spec (%)	PPV/RR (%)[a]	NPV (%)	AUC
ER IHC	>1	78	21	0	4	53	100.0	7.0	28.4	100.0	0.535
	>10	78	21	0	5	52	100.0	8.8	28.8	100.0	0.544
	>50	78	21	0	13	44	100.0	22.8	32.3	100.0	0.614
	>70	78	20	1	22	35	95.2	38.6	36.4	95.7	0.669
	>90	78	10	11	46	11	47.6	80.7	47.6	80.7	0.642
PR IHC	>1	79	22	0	10	47	100.0	17.5	31.9	100.0	0.588
	>10	79	22	0	20	37	100.0	35.1	37.3	100.0	0.675
	>50	79	22	0	35	22	100.0	61.4	50.0	100.0	0.807
	>70	79	18	4	48	9	81.8	84.2	66.7	92.3	0.830
	>90	79	3	19	56	1	13.6	98.2	75.0	74.7	0.559
ERPAS	>15	73	19	0	38	16	100.0	70.4	54.3	100.0	0.852

AUC, area under the curve; *ER*, estrogen receptor; *ERPAS*, estrogen receptor pathway activity score; *FN*, false negative; *FP*, false positive; *IHC*, immunohistochemical expression; *NPV*, negative predictive value; *PPV*, positive predictive value; *PR*, progesterone receptor; *RR*, response rate; *sens*, sensitivity; *spec*, specificity; *TN*, true negative; *TP*, true positive.

[a] By definition, PPV is equal to the RR (number of cases with a response divided by the number of cases with a positive test result).

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SUPPLEMENTAL TABLE 2
Correlation among hormonal biomarkers

Spearman rho correlation	ER IHC (%)	PR IHC (%)	ERPAS
ER IHC (%)	1		
PR IHC (%)	0.647[a]	1	
ERPAS	0.572[a]	0.786[a]	1
AR IHC (%)	0.162	0.320[a]	0.174

AR, androgen receptor; *ER*, estrogen receptor; *ERPAS*, estrogen receptor pathway activity score; *IHC*, immunohistochemical expression; *PR*, progesterone receptor.

[a] Significant correlation at the 0.01 level.

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