Precision Medicine and Imaging

Clinical Cancer Research

Risk Assessment after Neoadjuvant Chemotherapy in Luminal Breast Cancer Using a Clinicomolecular Predictor

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Abstract

Purpose: This study aimed to evaluate a modified EPclin test (mEPclin), a combination of EndoPredict (EP) score, post-neoadjuvant pathologic tumor size and nodal status, for predicting the risk of distance recurrence after neoadjuvant chemotherapy (NACT) in patients with residual estrogen receptor (ER)–positive/HER2-negative breast cancer. We also compared the prognostic power of the mEPclin with that of the CPS-EG score.

Experimental Design: A total of 428 formalin-fixed, paraffin-embedded tumor samples from GeparTrio and GeparQuattro studies were evaluated for mRNA expression of eight cancer-related and three reference genes. The mEPclin score was computed using a modified algorithm and predefined cut-off values were used to classify each patient at low or high risk. Primary endpoint was disease-free survival (DFS).

Results: A higher continuous mEPclin score was significantly associated with increased risk of relapse [HR, 2.16; 95% confidence interval (CI), 1.86–2.51; $P < 0.001$] and death (HR, 2.28; 95% CI, 1.90–2.75; $P < 0.001$). Similarly, patients classified at high risk by dichotomous mEPclin showed significantly poorer DFS and overall survival compared with those at low risk. In contrast with CPS-EG, the mEPclin remained significantly prognostic for DFS in multivariate analysis (HR, 2.13; 95% CI, 1.73–2.63; $P < 0.001$). Combining CPS-EG and other clinicopathological variables with mEPclin yielded a significant improvement of the prognostic power for DFS versus without mEPclin (c-indices: 0.748 vs. 0.660; $P < 0.001$).

Conclusions: The mEPclin score independently predicted the risk of distance recurrence and provided additional prognostic information to the CPS-EG score to assess more accurately the prognosis after NACT in the luminal non-pCR patient population. Therefore, this approach can be used to select patients for additional post-neoadjuvant therapies. *Clin Cancer Res; 24(14); 3358–65.* ©*2018 AACR.*

Introduction

The association between pathological complete response (pCR) defined as ypT0ypN0 or ypT0is/ypN0 and long-term outcome after neoadjuvant therapy has been shown to be the strongest in patients with aggressive tumors such as hormone receptor (HR)-negative/HER2-positive and triple-negative breast cancer (1). In the luminal-like breast cancer subtype, pCR was as strongly correlated with improved disease-free survival (DFS) as in other subtypes. Therefore, in operable luminal-like breast cancer new approaches for post-neoadjuvant chemotherapy (NACT) risk prediction are needed.

In the ER-positive/HER2-negative breast cancer subtype molecular tests such as Breast Cancer Index (BCI; ref. 2), IHC-based IHC4 test (3), and EndoPredict (EP; ref. 4) have been recently developed to determine the risk of recurrence after adjuvant endocrine therapy. None of these assays has been validated for assessing the risk of recurrence and death in ER-positive/HER2-negative breast cancer after NACT.

A staging system using pretreatment clinical stage (CS), ER status (E), grade (G), and post-treatment pathologic stage (PS; CPS-EG) has been developed to assess prognosis after NACT and to help selecting patients who could potentially benefit from further treatment. The CPS-EG score is able to

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

Prior presentation: Presented in part at the San Antonio Breast Cancer Symposium, December 9–13, 2014 and in the 52th Annual Meeting of the American Society of Clinical Oncology, June 3–7, 2016.

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doi: 10.1158/1078-0432.CCR-17-2947

Translational Relevance

Identifying patients who require additional therapy after neoadjuvant treatment is of increasing clinical relevance in patients with luminal-like breast cancer as many new treatment options for these patients are available or in development. In this study, we evaluated a modified EPclin test (mEPclin), a novel combination of post-neoadjuvant EndoPredict (EP) risk score, pathological tumor size, and nodal status for predicting survival of patients with residual ER-positive/HER2-negative breast cancer after neoadjuvant chemotherapy (NACT). The mEPclin score independently predicted the risk of distance recurrence and provided additional prognostic information to the CPS-EG score to assess more accurately the prognosis after NACT in the luminal non-pCR patient population. This novel approach using the mEPclin seems to be able to identify a cohort of patients with ER-positive/HER2-negative breast cancer at high risk for relapse after NACT, which might benefit from additional post-neoadjuvant therapies, for example, treatment with CDK4/6 inhibitor.

distinguish 7 prognostic subgroups of patients with significantly different 5-year distant metastasis-free and breast cancer–free survival, with a higher score associated with worse prognosis (5, 6). The prognostic value of the CPS-EG score has been further validated in a subgroup of patients with HR-positive/HER2-negative tumors treated with anthracycline/taxane-based NACT and confirmed its clinical utility to stratify patients in more refined prognostic subgroups than CS or PS alone (7). Recently, the CPS-EG staging system has been improved by incorporating a different cutoff value for ER positivity (1% vs. 10%) and the expression status of HER2 (Neo-Bioscore; ref. 8). Beside the CPS-EG score, there are no reliable biomarkers to identify the risk of recurrence after NACT in patients with ER-positive/HER2-negative breast cancer (9).

In the adjuvant setting, an EP score (12-gene molecular score) has been recently developed to predict the likelihood of distant recurrence in patients with ER-positive/HER2-negative breast cancer treated with adjuvant endocrine therapy only (4). It is a multigene expression signature consisting of eight cancer-related and three reference genes. The EP score was combined with the clinical parameters tumor size and nodal status into a comprehensive clinicomolecular risk score, EPclin. The EP and EPclin scores were further validated in prospective–retrospective studies performed on adjuvant settings of patients with ER-positive/HER2-negative tumors and confirmed their utility to provide additional prognostic information independent from clinicopathological parameters (4, 10–12). However, data on the application of the EPclin score in a neoadjuvant setting of patients with luminal breast cancer are lacking. To assess the risk of recurrence in patients with residual luminal breast cancer after NACT, we have modified the EPclin test by combining EP score with post-neoadjuvant pathological tumor size and nodal status (mEPclin). All cutoff values were predefined and identical to the cutoff values used for EPclin in the adjuvant setting. Our preliminary data showed that the mEPclin performed on a small cohort of patients with ER-positive/HER2-negative breast cancer, nonresponding to the NACT from GeparTrio study provided independent prognostic information and performed better than the CPS-EG score alone (13).

In this biomarker study, we used a larger cohort of patients from two randomized neoadjuvant studies (GeparTrio and GeparQuattro) to evaluate the mEPclin score for predicting the risk of distance recurrence in luminal breast cancer patients not achieving pCR after NACT. We also compared the prognostic power of the mEPclin with that of the CPS-EG score.

Material and Methods

Study design

Patients with centrally conformed ER-positive/HER2-negative breast cancer receiving NACT within the GeparTrio and GeparQuattro studies, not achieving a pCR and with available follow-up were considered. The GeparTrio (NCT00544765), a randomized phase III trial, compared four cycles of docetaxel, doxorubicin, and cyclophosphamide (TAC) with four cycles of vinorelbine and capecitabine in patients not sufficiently responding to two cycles of TAC and four cycles of TAC versus six cycles of TAC in patients sufficiently responding to two cycles of TAC as preoperative treatment of locally advanced (T4 a-d, N0-3, M0) or operable (T $\geq$ 2 cm, N0-2, M0) primary breast cancer (14, 15). In the GeparQuattro (NCT00288002), a randomized phase III trial, patients with operable or locally advanced breast cancer were treated preoperatively with or without capecitabine given concomitantly or in sequence to four cycles docetaxel following four cycles epirubicin/cyclophosphamide. Patients with HER2-positive disease received trastuzumab every 3 weeks during all chemotherapy cycles (16, 17). The trials were performed after approval by the Institutional Review Boards (IRB) and conducted in accordance with the International Ethical Guidelines for Biomedical Research involving Human Subjects (CIOMS). Written informed consent for study participation and biomaterial collection was obtained from all patients.

The ER, HER2, and Ki67 expression were centrally assessed by immunohistochemistry (IHC). ER positivity was defined as $\geq$10% of stained tumor cells and HER2 negativity was defined as immunohistochemistry (IHC) score 0, 1+ or 2+ without SISH amplification. The mEPclin was performed on residual tumor specimen (formalin-fixed paraffin embedded, FFPE tissue) with tumor area $\geq$ 25 mm^2 and tumor cell content $\geq$ 20%.

RNA extraction, gene expression analysis

Total mRNA was extracted from 10-μm tissue sections and assessed for expression of eight cancer-related genes (*BIRC5*, *RBBP8*, *UBE2C*, *IL6ST*, *AZGP1*, *DHCR7*, *MGP*, *STC2*) and three reference genes (*CALM2*, *OAZ1*, *RPL37A*) by quantitative reverse transcriptase PCR (qRT-PCR) analysis (14). The laboratory personnel performing the gene expression analysis was blinded to clinicopathological characteristics and therapy response. As previously described (4), the EP score was calculated as a linear combination of the delta cycle threshold (ΔC_t) values of the eight cancer-related genes based on the normalization on the average of the three reference genes.

Assignment to CPS-EG and EPclin scores

The CPS-EG score was assessed as previously described (6). The EPclin score (4) was defined as a linear combination of the baseline EP score, clinical tumor size- and clinical nodal status, where the tumor size was coded as T1a-b = 1, T1c = 2, T2 = 3, and

T3 = 4, and the nodal status was coded as N0 = 1, 1–3 positive nodes = 2, 4–10 positive nodes = 3, and >10 positive nodes = 4. As the GeparTrio and GeparQuattro post-NACT parameters did not allow the same coding, we used a modified EPclin (mEPclin) score for our analyses. The mEPclin score was defined as a linear combination of the post-NACT EP score, post-NACT tumor size, and post-NACT nodal status, where the linear coefficients were the same as for the EPclin score, but the tumor size and the nodal status were coded as: ypT1 = 1.5; ypT2 = 3; ypT3 = 4; ypT4 = 4; ypN0 = 1; ypN1 (1–3 positive nodes) = 2; ypN2 (4–9 positive nodes) = 3; ypN3 (≥10 positive nodes) = 4. Patients were stratified into low or high risk of distant recurrence according to the same cutoff level as for the EPclin (4): the mEPclin score <3.32867 was defined as low risk and ≥3.32867 as high risk. The CPS-EG score <3 was defined as low risk and ≥3 as high risk.

Outcomes

Primary endpoint was DFS defined as the time from randomization to any invasive loco-regional, contralateral or distant recurrence of breast cancer or any second primary invasive non-breast cancer or death due to any cause. Secondary endpoint was overall survival (OS) defined as the time from randomization to death due to any cause.

Statistical analysis

Differences in clinicopathological characteristic between the groups were assessed using the Mann–Whitney *U* test for continuous variables and the Fishers exact (two classes) or χ^2 test (three or more classes) for categorical variables. DFS and OS rates were estimated using the Kaplan–Meier method. Uni- and multivariate Cox regressions were used to assess hazard ratios (HR) with 95% symmetric confidence intervals (CIs). For continuous variables the HRs referred to one unit increase of the variable. To assess the mEPclin prognostic power c-index estimates were performed; in case of multivariate calculations cross validation and permutation test were used, thus the estimates and *P* values were unbiased. Values of $P < 0.05$ were considered statistically significant.

Results

The mEPclin score was evaluated in 428 patients with available follow-up and residual ER-positive/HER2-negative breast cancer after NACT. Of them, the CPS-EG score was assessed in 418 patients, 10 patients were excluded due to not available baseline nodal status or tumor grade (Fig. 1, Consort). Baseline clinicopathological characteristics are presented in Table 1.

After a median follow-up of 67.4 months (range, 2.8–154.0 months) a higher continuous mEPclin score was associated with a significantly shorter DFS (HR, 2.16; 95% CI, 1.86–2.51; $P < 0.001$) and OS (HR, 2.28; 95% CI, 1.90–2.75; $P < 0.001$; Table 2).

Patients were classified into a low-risk and high-risk group according to the predefined cutoff values of the dichotomous mEPclin or CPS-EG variables. Both DFS (HR, 4.22; 95% CI, 2.57–6.92; $P < 0.001$) and OS (HR, 3.66; 95% CI, 2.00–6.69; $P < 0.001$) were significantly worse in the high mEPclin risk group compared with the low-risk group (Fig. 2A). Similarly, the group with high CPS-EG score had a significantly worse DFS (HR, 2.95; 95% CI, 2.02–4.33; $P < 0.001$) and OS (HR, 3.70; 95% CI, 2.29–5.99; $P < 0.001$; Fig. 2B) compared with the low-risk group.

Bivariate Cox regression analyses showed that the higher continuous mEPclin risk score remained significantly prognostic for the reduced DFS (HR, 2.09; 95% CI, 1.73–2.53; $P < 0.001$) and OS (HR, 2.16; 95% CI, 1.72–2.72; $P < 0.001$) compared with the CPS-EG score (Table 2). Multivariate analyses for DFS confirmed the significant prognostic value of the mEPclin score compared with the CPS-EG score after adjustment for clinicopathological characteristics (Table 3). Similar results were obtained by adding Ki67 to the multivariate model (Supplementary Table S1). It

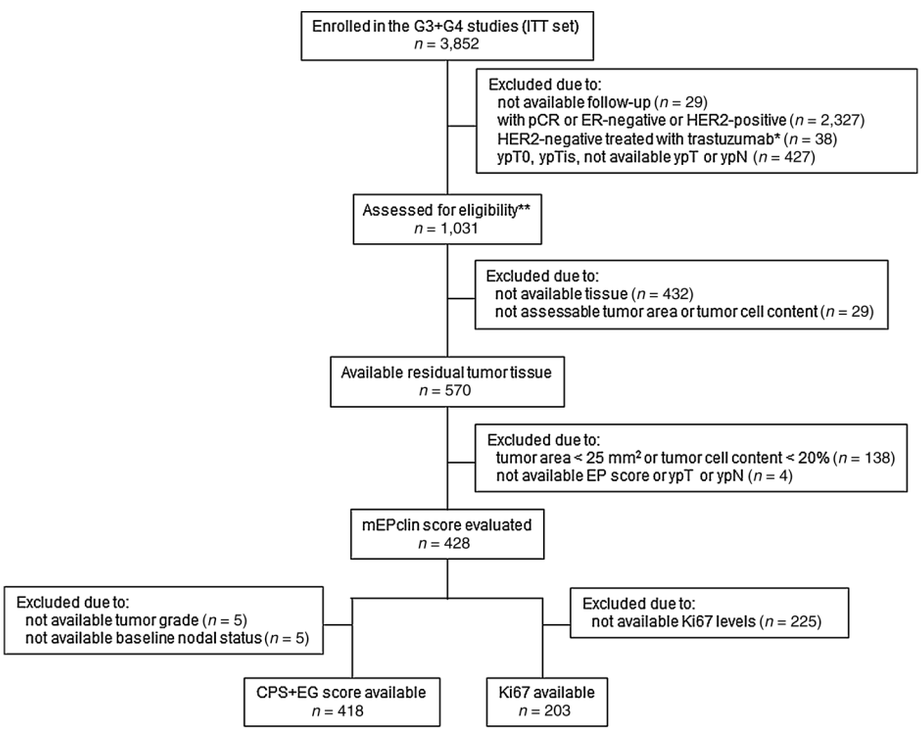

Figure 1.
Consort diagram of the availability of samples for analysis. pCR, pathological complete response; ypN, pathological nodal status; ypT, pathologic tumor size; *, Patients locally assessed HER2-positive but centrally HER2-negative; **, Eligibility criteria were: no pCR, ER-positive/HER2-negative breast cancer, no trastuzumab treatment, available ypT, and available ypN.

Table 1. Clinicopathologic characteristic of patient cohort

Characteristics	Category	Samples analyzed ($n = 428$) *N* (%)	Overall ($n = 1,031$) *N* (%)	*P*
Study	GeparTrio	257 (60.0)	756 (73.3)	<0.001
	GeparQuattro	171 (40.0)	275 (26.7)	
Age	<50	188 (43.9)	458 (44.4)	0.799
	≥50	240 (56.1)	573 (55.6)	
Grading at baseline	1	23 (5.4)	66 (6.6)	0.243
	2	315 (74.5)	723 (71.9)	
	3	85 (20.1)	217 (21.6)	
	Unknown	5	25	
Clinical tumor stage	T1	5 (1.2)	8 (0.8)	0.361
	T2	277 (64.7)	683 (66.3)	
	T3	80 (18.7)	183 (17.8)	
	T4a-c	41 (9.6)	107 (10.4)	
	T4d	25 (5.8)	49 (4.8)	
	Unknown	0	1	
Clinical nodal stage	Negative	188 (44.4)	487 (47.8)	0.115
	N1-3	212 (50.1)	488 (47.9)	
	N4-9	18 (4.3)	31 (3.0)	
	N≥10	5 (1.2)	12 (1.2)	
	Unknown	5	13	
Post-surgery tumor stage	ypT1	187 (43.7)	523 (50.7)	0.001
	ypT2	157 (36.7)	351 (34.0)	
	ypT3	61 (14.3)	111 (10.8)	
	ypT4a-c	20 (4.7)	42 (4.1)	
	ypT4d	3 (0.7)	4 (0.4)	
Post-surgery nodal status	ypN0	161 (37.6)	462 (44.8)	<0.001
	ypN1-3	140 (32.7)	320 (31.0)	
	ypN4-9	75 (17.5)	171 (16.6)	
	ypN≥10	52 (12.1)	78 (7.6)	
CPS-EG (dichotomous)	Low	285 (68.2)	732 (73.8)	0.001
	High	133 (31.8)	260 (26.2)	
	Missing	10	39	
mEPclin (dichotomous)	Low	175 (40.9)		n.a.
	High	253 (59.1)		
	Missing	0		
CPS-EG (continuous)	Median [range]	2 [0–5]	2 [0–5]	0.001
mEPclin (continuous)	Median [range]	3.6 [1.6–7.6]		n.a.
Baseline Ki67 (continuous)	Median [range]	15 [0–86]	16 [0–95]	0.347

should be noted that the Ki67 levels were available for only 203 tumor samples; of them 198 with also available CPS-EG score were included in the multivariate analysis. Comparison between the mEPclin and CPS-EG performance showed that 146 (34.9%) of 418 samples were classified discordantly as at low or at high risk. No statistically significant difference was observed for DFS and OS in both discordant subgroups, mEPclin low/CPS-EG high versus mEPclin high/CPS-EG low subgroup (DFS HR, 0.72; 95% CI, 0.22–2.35; $P = 0.592$ and OS HR, 1.01; 95% CI, 0.23–4.37; $P = 0.989$; Supplementary Fig. S1). Within the low-risk CPS-EG group mEPclin was able to classify 131 (46.0%) of 285 patients as high risk with significantly worse DFS (HR, 3.31; 95% CI, 1.84–5.96; $P < 0.001$) and OS (HR, 2.23; 95% CI, 1.05–4.74; $P = 0.036$) compared with the low-risk mEPclin subgroup of patients. Within the high-risk CPS-EG group the separation between mEPclin high- and low-risk subgroups did not show significant differences for DFS and OS (Fig. 3).

The addition of the mEPclin score to a combination of parameters, including CPS-EG score, study (GeparQuattro vs. GeparTrio), age, and tumor grade resulted in a significant improvement of the prognostic power for DFS compared with the clinicopathological model alone [concordance indices (c-indices): 0.748 with mEPclin vs. 0.660 without mEPclin; $P < 0.001$]. Similar results were obtained by combining mEPclin score with the Ki67, CPS-EG score, age and tumor grade (c-indices: 0.752 with mEPclin vs. 0.725 without mEPclin; $P = 0.001$; Supplementary Fig. S2). Interestingly, the mEPclin score alone

Table 2. Univariate and bivariate Cox regression—comparison of the continuous mEPclin and CPS-EG scores

Parameter	Univariate Cox regression					Bivariate Cox regression				
	DFS			OS		DFS			OS	
	N	HR (95% CI)	*P*	HR (95% CI)	*P*	*N*	HR (95% CI)	*P*	HR (95% CI)	*P*
mEPclin	428	2.16 (1.86–2.51)	<0.001	2.28 (1.90–2.75)	<0.001	418	2.09 (1.73–2.53)	<0.001	2.16 (1.72–2.72)	<0.001
CPS-EG	418	1.70 (1.43–2.02)	<0.001	1.81 (1.47–2.24)	<0.001	418	1.06 (0.85–1-31)	0.616	1.12 (0.86–1.46)	0.393

Abbreviation: *N*, number of patients.

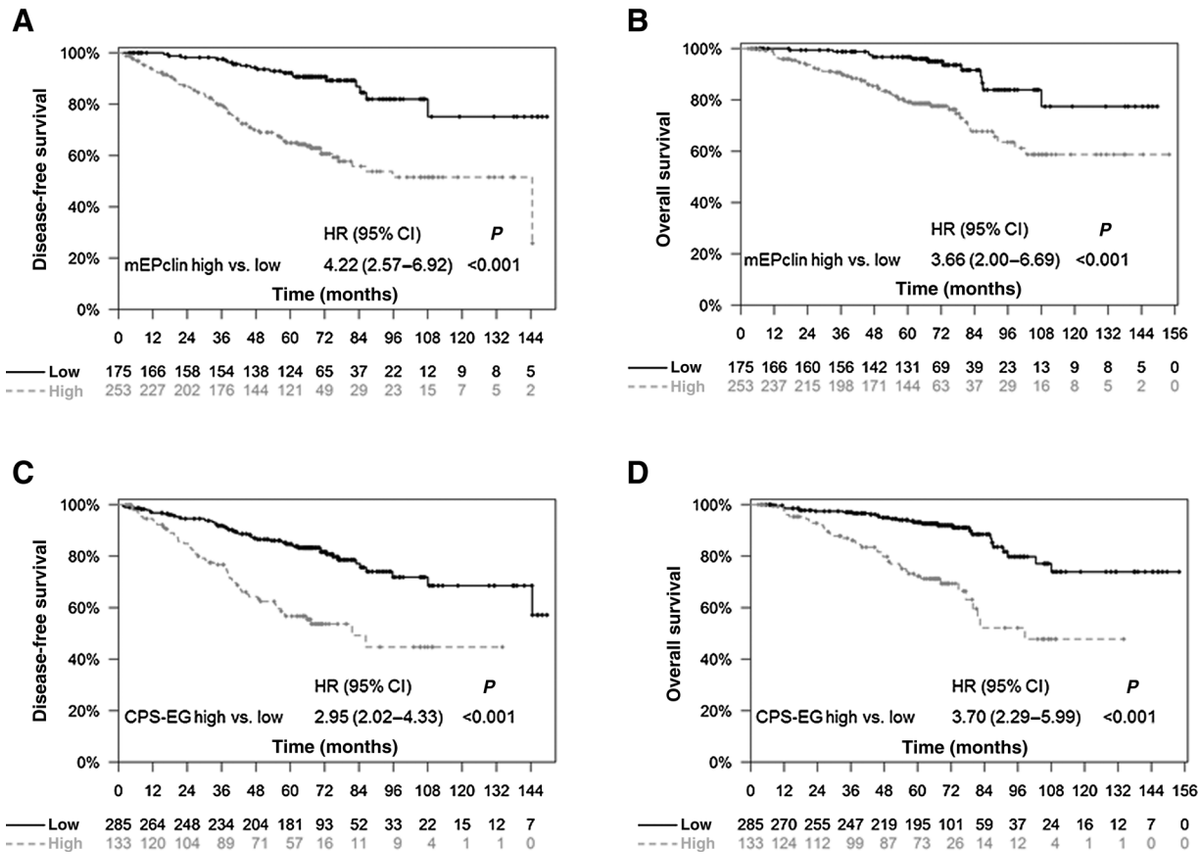

Figure 2.

Kaplan–Meier plots for DFS and OS according to dichotomous mEPclin (**A–B**) and CPS-EG (**C–D**) scores in patients with ER-positive/HER-negative breast cancer not achieving pCR after NACT. Curves (gray, high-risk group; black, low-risk group) represented estimated DFS for mEPclin high- versus mEPclin low-risk group of patients ($n = 428$; **A**); OS for mEPclin high versus mEPclin low-risk group of patients ($n = 428$; **B**); DFS for CPS-EG high- versus GPS-EG low-risk group of patients ($n = 418$; **C**); OS for CPS-EG high- versus CSP-EG low-risk group of patients ($n = 418$; **D**).

showed higher prognostic values (c-index 0.758) compared with the combinations of clinicopathologic and molecular parameters above.

Discussion

Identifying patients who require additional therapy after neoadjuvant treatment is of increasing clinical relevance in patients with luminal-like breast cancer as many new treatment options for these patients are available or in development.

In this study, we showed that the mEPclin score can be used to predict the long-term outcome in patients with residual ER-positive/HER2-negative breast cancer after NACT from two prospective randomized neoadjuvant trials. Furthermore, a direct comparison between mEPclin and CPS-EG showed that the mEPclin score was superior especially in the subgroup of patients classified as low risk by the CPS-EG score.

The prognostic utility of CPS-EG staging system has been validated in patients with HR-positive/HER2-negative breast cancer (7) and recently used as an inclusion criterion in the PENELOPE-B clinical trial (NCT01864746), a phase III post-neoadjuvant study evaluating palbociclib in patients with HR-positive/HER2-normal primary breast cancer with high risk of relapse after NACT. The CPS-EG score included routinely

Table 3. Multivariate comparison of mEPclin and CPS-EG for DFS

		Multivariate analysis ($n = 418$)	
Parameters	**References**	**HR (95% CI)**	***P***
mEPclin	Continuous	2.13 (1.73–2.63)	<0.001
CPS-EG	Continuous	1.04 (0.81–1.35)	0.741
ypN+	ypN0	1.10 (0.62–1.98)	0.739
ypT3-4	ypT1-2	0.90 (0.55–1.45)	0.651
Age ≥50	Age <50	0.96 (0.65–1.42)	0.848
Grade 3	Grade 1-2	1.10 (0.65–1.86)	0.720
GeparQuattro	GeparTrio	0.76 (0.51–1.15)	0.195

Abbreviations: ypN, pathologic nodal status; ypT, pathologic tumor size.

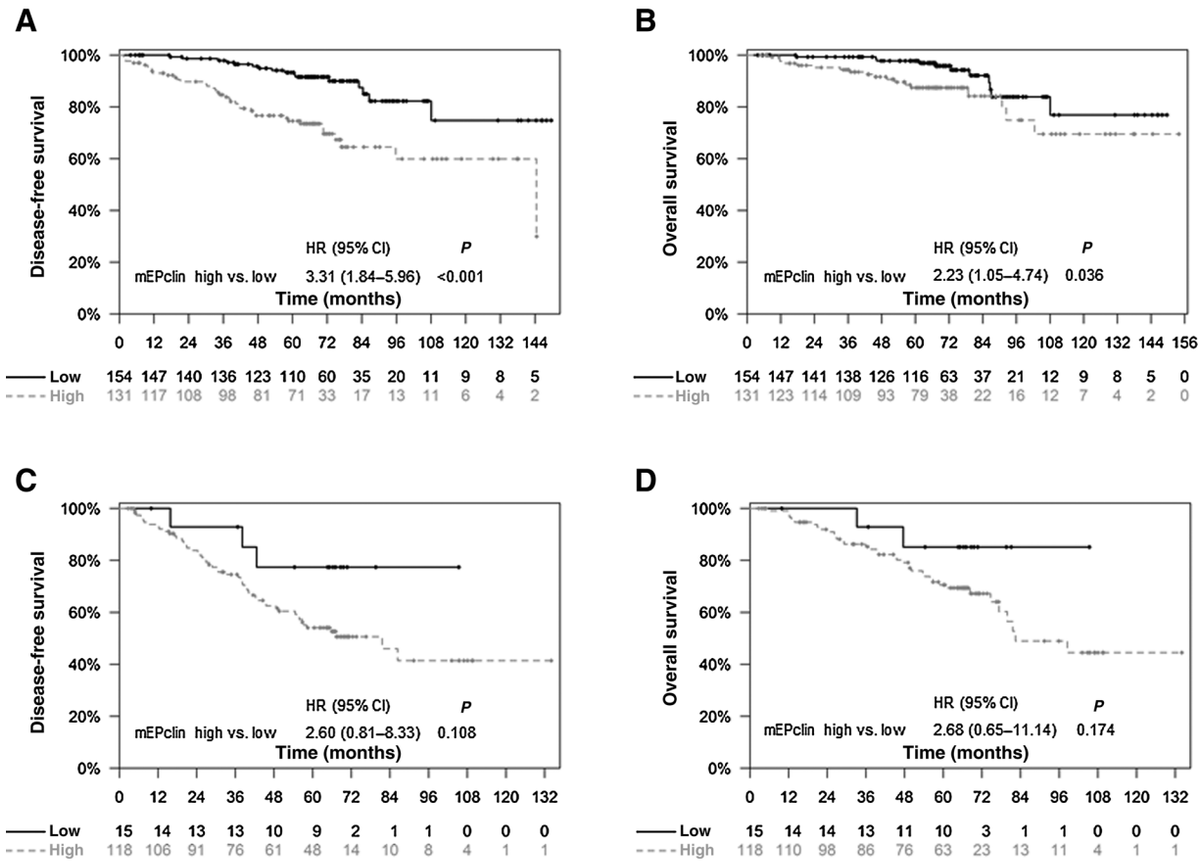

Figure 3.

Kaplan–Meier plots for DFS (**A**) and OS (**B**) according to mEPclin in low-risk group stratified by CSP+ER score and for DFS (**C**) and OS (**D**) according to mEPclin in high-risk group stratified by CSP+ER score. Curves (gray, high-risk group; black, low-risk group) represented estimated DFS for mEPclin high- versus mEPclin low-risk subgroup of patients who were stratified as low CPS-EG risk group ($n = 418$; **A**); OS for mEPclin high- versus mEPclin low-risk subgroup of patients who were stratified as low CPS-EG risk group ($n = 418$; **B**); DFS for mEPclin high versus mEPclin low-risk subgroup of patients who were stratified as high CPS-EG risk group ($n = 418$; **C**); and OS for mEPclin high versus mEPclin low-risk subgroup of patients who were stratified as high CPS-EG risk group ($n = 418$; **D**).

reported pretreatment clinical and post-treatment pathological parameters at no additional costs supporting its wide availability, whereas the mEPclin score requires additional molecular testing. However, our mEPclin model facilitated a more precise assessment of long-term outcome within the low-risk subgroup of patients with ER-positive/HER2-negative breast cancer as compared with the CPS-EG score, and therefore improved the categorization of those patients into more refined prognostic subgroups.

Furthermore, the relatively high number of patients with intermediate-grade tumors (G2, 74.5%) included in our study suggested that mEPclin can be used to predict the risk of recurrence in this subgroup of breast cancer patients who usually show a better long-term prognosis compared with the higher-grade HR-positive/HER2-negative breast cancer (1, 18, 19).

The integration of tumor molecular characteristics with the post-NACT clinicopathological parameters can improve the stratification of prognosis in patient with and without pCR. A variate of studies aiming to identify reliable tools for assessing risk of recurrence after NACT included evaluation of residual tumor not only by measuring the pathological tumor stage but also assessing the residual cancer burden (RCB) and tumor biology. Recently, the RCB has been in focus as another approach for assessing the long-term prognosis after NACT in different breast cancer subtypes (20). The RCB index score combined the largest area of the tumor bed, the cellularity of residual invasive tumor, the number of involved lymph nodes and the size of the largest nodal metastasis. In all except the HR-positive/HER-negative breast cancer subtype, prognosis for patients with pCR (RCB-0, ypT0/is ypN0) was better than for those with minimal RCB (RCB-I). In patients with luminal breast cancer the RCB score was only prognostic in node positive tumors (20). However, the prognostic information derived from the RCB score was limited as no pretreatment information was included compared with the CPS-EG as well as no molecular tumor characteristics compared with the mEPclin.

The preoperative endocrine prognostic index (PEPI) for recurrence-free survival (RFS) in ER-positive breast cancer included tumor size, nodal status, Ki67 levels and ER status after neoadjuvant endocrine therapy, has also been shown to provide independent prognostic information (21). It could be used to select patients at an extremely low risk for recurrence (PEPI score 0) who may not benefit from additional adjuvant chemotherapy. The PEPI approach was incorporated in the neoadjuvant trial

ACOSOG Z1031A designed to determine which of the three different aromatase inhibitors (exemestane, letrozole, and anastrozole) could be chosen for future treatment of patients with ER-positive breast cancer (22). The trial was amended (ACOSOG Z1031B) to allow a switch to neoadjuvant chemotherapy in patient with a tumor Ki67 value greater than 10% after 2 to 4 weeks of neoadjuvant endocrine therapy. The relapse risk over 5 years in those patients who were categorized with PEPI score 0 and did not received adjuvant chemotherapy was only 3.7% compared with 14.4% in patients with a PEPI score >0 (23). However, more prospective validation studies with larger sample size and improved reproducibility of Ki67 evaluation are warranted to confirm the PEPI score as a tool for adjuvant treatment individualization after neoadjuvant endocrine therapy (24).

Posttreatment Ki67 levels have also been reported to provide independent prognostic information beyond pCR in patients with residual HR-positive breast cancer after NACT but the Ki67 levels were not prognostic for outcome after response-guided chemotherapy (25).

We showed that the mEPclin alone as well as its addition to the combination of Ki67, CPS-EG and other clinicopathological parameters provided significant independent prognostic information in luminal non-pCR breast cancer patients after NACT according to outcome.

The strengths of our study were inclusion of a homogenous breast cancer cohort consisting of only ER-positive/HER2-negative patients from two prospective neoadjuvant trials, and thus minimized confounding effects from other tumor subtypes. The use of predefined and validated gene-expression panel and algorithm design (4) allowed precise assessment of the mEPclin score. Moreover, this report is the first direct comparison of the mEPclin score with CPS-EG score. Despite these valuable strengths, our study has some limitations. The sample size did not enable us to detect clear moderate differences in prognosis between the discordant subgroups. Another limitation was the small number of patients with evaluable Ki67 levels. Hence, further prospective validation studies with larger sample sizes are warranted to confirm the mEPclin as a tool for selecting patients with ER-positive/HER2-negative breast cancer in need for post-neoadjuvant treatment. However, these limitations did not influence the significant prognostic power of the mEPclin score compared with CPS-EG score. Furthermore, the mEPclin seems to be able to identify a cohort of patients at high risk for relapse after NACT that was classified at low risk by the CPS-EG score. Thus, this high-risk mEPclin subgroup of patients with residual ER-positive/HER2-negative breast cancer might benefit from additional post-neoadjuvant therapy.

In conclusion, the mEPclin score independently predicted the risk of distance recurrence and provided additional prognostic information to the CPS-EG score. Therefore, this novel approach can be used to assess more accurately the prognosis after NACT in the luminal non-pCR patient population and to select only those patients who might benefit from additional post-neoadjuvant therapies, for example, treatment with CDK4/6 inhibitor.

Disclosure of Potential Conflicts of Interest

S. Loibl reports receiving commercial research support from Sividon Diagnostics. K. Weber has ownership interests (including patents) at Sividon Diagnostics, and receives inventor's remuneration for EndoPredict. J. Huober reports receiving speakers bureau honoraria from Celgene, Novartis, and Roche, and is a onsultant/advisory board member for Amgen, Celgene, Novartis and Roche. S. Kümmel is a consultant/advisory board member for Amgen, Celgene, Daiichi Sankyo, Novartis, and Roche. V. Müller is a consultant/advisory board member for Genomic Health. R. Kronenwett and C. Denkert have ownership interests (including patents) at Sividon Diagnostics. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

Dr. Valentina Vladimirova provided editorial support. This work was in part supported by the German Cancer Aid grant TransLUMINAL-B, Nr 111536.

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Received October 9, 2017; revised February 6, 2018; accepted March 29, 2018; published first April 4, 2018.

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