

## **Prognostic Value of EndoPredict in Women with Hormone Receptor Positive, HER2-Negative Invasive Lobular Breast Cancer**

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**Running title:** Risk prediction by EndoPredict for ILC

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### **Translational relevance**

Risk of recurrence has been traditionally evaluated using standard clinic-pathological factors, such as nodal status, tumour size, tumour grade, age, and immune-histochemical markers. Multigene assays are more frequently used to better determine prognosis and treatment options in women with hormone receptor positive, HER2-negative breast cancer. Most of these multigene assays have been developed in women with invasive ductal carcinoma or of unknown histological subtypes. Women with invasive lobular carcinoma have specific disease characteristics and outcomes vary by histological subtypes. It is important to understand whether these multigene assays perform equally well in both subtypes and it is therefore necessary to evaluate the prognostic performance in this specific subtype.

## Abstract

**Purpose:** Invasive lobular carcinoma (ILC) accounts for approximately 5-15% of all invasive breast cancer cases. Most of the correlations between multigene assays and patient outcome were derived from studies based on patients with invasive ductal carcinoma (IDC) or without distinction between the subtypes. Here, we investigate the prognostic value of EndoPredict in a large cohort of ILCs pooled from three phase III randomized trials (ABCSG-6, ABCSG-8, TransATAC).

**Experimental design:** The primary objective of this analysis was to determine the prognostic value of EndoPredict (EPclin) for distant recurrence in years 0-10 in postmenopausal women with ILC. The primary outcome was distant recurrence (DR).

**Results:** 470 women (17.9%) presented with ILC, 1944 (73.9%) with IDC, and 216 (8.2%) had other histological types. EPclin was highly prognostic in women with ILC (HR=3.32 (2.54-4.34)) and provided more prognostic value than the Clinical Treatment Score (CTS) (HR=2.17 (1.73-2.72)). 63.4% women were categorised into the low EPclin risk group and they had a 10-year DR of 4.8% (2.7-8.4) compared to 36.6% of women in the high risk group with a 10-year DR risk of 26.6% (20.0-35.0). EPclin also provided highly prognostic information in women with node-negative disease (HR=2.56 (1.63-4.02)) and node-positive disease (HR=3.70 (2.49-5.50)).

**Conclusions:** EPclin provided highly significant prognostic value and significant risk stratification for women with ILC. 10-year DR risk in the EPclin low risk groups were similar between ILC and IDC. Our results show that EPclin is informative in women with ILC and suggest that it is equally valid in both histological subtypes.

## Introduction

Invasive breast cancer is a heterogeneous disease with specific morphologies and phenotypic features [1]. The majority of breast cancers are invasive ductal carcinoma (around 80%) (IDC), followed by invasive lobular carcinoma (5-15%) (ILC) and less than around 8% are tumours with other specific or special histological features (apocrine, medullary, mucinous, papillary). ILC tumour cells are typically round, small, relatively uniform, non-cohesive and have a characteristic growth pattern characterised histologically as single-file infiltration of the stroma. Inactivation of E-cadherin (*CDH1*) by mutation, loss of heterozygosity, or methylation are characteristic molecular changes in ILC, particularly the pleomorphic subtype [2]. Outcome varies by histological subtypes, with ILC typically presenting with favourable features such as associated with good prognosis, such as oestrogen receptor (ER) positivity, low to intermediate grade, low Ki67 expression, and absence of *HER2* amplification [3].

Around 85% of women with ER-positive invasive disease remain recurrence free after 10-years, suggesting that the majority of patients are over-treated. Several multigene assays have been evaluated for the prognostication of early stage invasive ER-positive breast cancer and have shown clinical utility for the likelihood of risk of recurrence [4-8]. However, the development and validation of these assays have been mostly performed in women without considering histological subtype and little is known about the performance of these assays specifically in ILCs. EndoPredict (EPclin) covers several cellular processes, such as proliferation, apoptosis, DNA repair, cell adhesion and cell signaling and combines the expression of proliferative and ER-signalling associated genes with information on nodal status and tumour size [9-11]. This combined algorithm is used as the diagnostic test in the clinical setting. EPclin has been validated as a prognostic test in pre- and postmenopausal women with ER-positive, HER2-negative breast cancer [6, 10, 12, 13]. More recently, EPclin has been evaluated for the prediction of chemotherapy benefit in a combined analysis of 5 large clinical trials [14].

Here, we evaluate the ability of EPclin to predict distant recurrence at 10 years specifically in women with ILC and compare the prognostic performance to women with IDC.

## Methods

2630 postmenopausal women with oestrogen receptor (ER) positive, HER2-negative breast cancer from three large clinical trials (ABCSG-6, ABCSG-8, TransATAC) were included in this analysis. All received 5 years of endocrine treatment as the only adjuvant therapy. The ABCSG-6 trial (ClinicalTrials.gov Identifier: NCT00309491) and ABCSG-8 trial (ClinicalTrials.gov Identifier: NCT0029175) included postmenopausal women with hormone receptor positive breast cancer who received five years of tamoxifen alone or five years of tamoxifen plus aminoglutethimide for the first 2 years [15] or five years of tamoxifen or two years of anastrozole followed by three years of tamoxifen [16], respectively. Women in the TransATAC trial (ClinicalTrials.gov Identifier: NCT00849030) were postmenopausal and received five years of tamoxifen or anastrozole alone [17]. Institutional review boards approved the individual studies, which were done in accordance with the Declaration of Helsinki (1996 revision). All women included in this analysis gave written informed consent.

The EndoPredict (EPclin) was developed in pre- and postmenopausal women with ER-positive, HER2-negative breast cancer [10]. For the TransATAC cohort, EP molecular scores were generated by qRT-PCR gene analysis by Myriad Genetics, Inc. For ABCSG-6 and 8 cohorts, EP molecular analysis was carried out by the ABCSG research group. All parties were blinded to clinical outcome data. As previously described [14], the EP molecular score incorporates the expression of eight cancer related genes (*BIRC5*, *UBE2C*, *DHCR7*, *RBBP8*, *IL6ST*, *AZGP1*, *MGP*, and *STC2*), three housekeeping genes (*CALM2*, *OAZ1*, and *RPL37A*), and one control gene (HBB). The EPclin incorporates information on nodal status and tumour size and higher EPclin scores indicate a higher risk of distant recurrence. Patients with an EPclin score of less than 3.3 are considered low risk whereas those with an EPclin of higher or equal to 3.3 are high risk [10]. We used the Clinical Treatment Score (CTS [18]) to assess the additional prognostic value of EPclin to clinical parameters. In brief, the CTS contains information on nodal status, tumour size, grade, age and treatment (tamoxifen vs. anastrozole).

Central review of lobular morphology was done in TransATAC (including sub-specification of lobular subtypes) and ABCSG-6 (morphology and E-Cadherin testing). In ABCSG-8, no central review was performed. However, lobular breast cancers were defined either by morphology alone or in most cases in combination with E-cadherin testing by a pathologist. For the present analysis, women with ILC or IDC histological subtypes were included (N=2414). Rare subtypes, such as tubule-lobular, pleomorphic, and solid, were not the focus of this analysis (N=216).

## Statistical Analysis

The primary endpoint was DR, defined as the time from randomisation in the primary study to DR of breast cancer. Local recurrence, regional recurrence, contralateral second primary or secondary breast cancer in the ipsilateral breast were not considered as DR. Deaths due to breast cancer without DR occurring prior to death were also considered as a DR at the time of death. Other causes of deaths were not considered as an event. All analyses were censored at 10 years of follow-up.

The primary objective of this analysis was to determine the prognostic value of EPclin, alone or in combination with clinical parameters, for DR in women with ILC in the combined dataset. Secondary objectives included differential risk stratification by EPclin in women with ILC, subgroup analyses in node-negative and node-positive ILC patients separately, prognostic value of EPclin for the risk of late DR in women with ILC, and comparison of prognostic value of EPclin between women with ILC versus IDC.

A predefined statistical analysis plan was approved by all research groups prior to data merge and data analyses. 10-year distant recurrence risks were assessed using Cox proportional hazard models. To assess prognostic performance of EPclin and clinical parameters, partial likelihood ratio (LR  $\chi^2$ ) tests and hazard ratios (HR) with associated 95% confidence intervals (CI) based on Cox proportional models were used. To compare the prognostic performance of EPclin and CTS, scores were normalised to have unit variance. The assumption of proportional hazards was assessed by Schoenfeld residuals. The improvement of DR in prognostication by EPclin compared with clinical and pathologic variables (CTS [18]) was quantified by the increase in the LR  $\chi^2$  value ( $\Delta$ LR  $\chi^2$ ; 2-sided 5% significance level). Kaplan-Meier estimates were used to estimate mean 10-year DR risks in pre-defined risk groups. All statistical analyses were two-sided, and a P value of less than .05 was regarded as significant. All analyses were performed with Stata software (version 15.1; StataCorp).

## Results

We had data for 2630 postmenopausal women with ER-positive, HER2-negative breast cancer. Of these 470 (19.5%) had ILC, 1944 (80.5%) had IDC, and the remaining 216 (8.2%) had other histological subtypes. Looking at the distribution of ILC by trial, 17.5% of women in ABCSG-6 had ILC, 20.7% in ABCSG-8, and 14% in TransATAC. Significantly more women in the ABCSG-8 trial had ILC compared to women in the TransATAC study ( $P<0.001$ ). Table 1 shows baseline characteristics by histological subtypes for the analysis cohort. In brief, women with ILC had significantly larger tumour size ( $P<0.001$ ), less differentiated tumours ( $P<0.001$ ), and higher CTS scores ( $P<0.001$ ) than women with IDC. Age, number of nodes involved, and EPclin scores were similar between the two subgroups (Table 1). For 22% of women with ILC, grade was not determined and therefore the CTS could not have been calculated. The median follow-up between the three trials was very similar and not significantly different: ABCSG-6: 9.02 years (IQR 5.0-11.5); ABCSG-8: 9.10 years (IQR 5.8-11.5); TransATAC: 9.9 years (IQR 7.7-10.1).

### Invasive lobular carcinoma (ILC)

EPclin was highly prognostic in women with ILC (HR=3.32 (2.54-4.34),  $P<0.0001$ ) (Table 2). EPclin provided significantly more prognostic value for DR in women with ILC than the CTS ( $\Delta\text{LR-}\chi^2=17.60$  ( $P<0.0001$ )) (Table 2). 298 women (63.4%) were categorised as low risk by EPclin compared to 172 (36.6%) who were deemed high risk (Figure 1). Women in the low EPclin risk group had a 10-year DR risk of 4.8% (2.7-8.4) compared to a 10-year DR risk of 26.6% (20.0-35.0) for women categorised into the high risk group. This translates to an over 6 times higher risk of developing a DR for women in the high EPclin risk group compared to the low EPclin risk group (HR=6.33 (3.31-12.12),  $P<0.0001$ ) (Figure 1A).

In women with node-negative disease, EPclin provided significant prognostic value for DR (HR=2.56 (1.63-4.02),  $P<0.0001$ ) whereas CTS did not provide any significant prognostic value (Table 2). Almost 80% of women (N=260) were categorised as low risk by EPclin with a 10-year DR risk of 4.6% (2.5-8.4) (Figure 1B). Those categorised into the high EPclin risk group (N=66 (20.3%)), had a 4.4-fold increased risk in developing a DR (HR=4.42 (1.84-10.65),  $P=0.001$ ) and a significantly higher 10-year DR risk (19.1% (10.6-33.3)) compared to those in the low risk group. In women with node-positive disease (N=144), 28 women (26.4%) were categorised as low risk by EPclin with the remaining women as high risk (73.6%). Significantly higher 10-year DR risks were observed for those in the EPclin high risk group compared to those in the low risk group (31.2% (22.4-42.3) vs. 6.4% (1.6-23.5))

(Figure 1C). In both nodal subgroups, EPclin provided significantly more prognostic information than the CTS alone (Table 2).

EPclin was also highly prognostic for late DR in 397 women with ILC who were recurrence-free 5 years after diagnosis (HR=3.83 (2.43-6.04),  $P<0.0001$ ) (Table 2). Compared to the CTS alone, EPclin provided significantly more prognostic information for late DR ( $\Delta\text{LR-}\chi^2=21.38$  ( $P<0.0001$ )), indicating a better prognostication for these late events. Two thirds of women were categorised to the low risk group with a 5-10 year DR risk of only 2.4% (1.0-5.8) compared to those 135 (34.0%) of women categorised into the high EPclin risk group who had a 10-year risk of 16.1% (10.2-24.7).

We also investigated the molecular EP score as a prognostic marker and observed overall similar but weaker effect sizes than for the EPclin. In all women with ILC, molecular EP was highly prognostic (HR=2.08 (1.65-2.64),  $P<0.0001$ ) and provided significant additional prognostic value to the CTS ( $\Delta\text{LR-}\chi^2=10.45$ ). For those with node negative disease (N=326), a 2.2-fold increase with every SD increase in molecular EP was observed (HR=2.19 (1.56-3.08),  $P<0.0001$ ). Similarly, in those with node-positive disease (N=144) molecular EP was highly prognostic (HR=2.31 (1.57-3.39),  $P<0.0001$ ) and provided additional prognostic value to the CTS ( $\Delta\text{LR-}\chi^2=5.64$ ).

#### Comparison between ILC and IDC

EPclin was highly prognostic in women with IDC (N=1944) overall (HR=2.36 (2.11-2.65),  $P<0.0001$ ), and in all subgroups (Figure 2). Overall, EPclin was more prognostic in ILC than IDC, with significant heterogeneity between the two subtypes ( $P=0.045$ ). For sub-groups wider confidence intervals were observed due to fewer women with ILC and fewer DR events compared to women with IDC, and no significant heterogeneity was observed. EPclin categorised non-significantly more women with ILC (63.4%) as low risk compared to those with IDC (59.1%). 10-year DR risks for low and high risk groups by EPclin were similar for those with ILC versus IDC (low risk: 4.8% vs. 5.4% and high risk: 26.6% vs. 23.5%, respectively).

Although special subtypes were not the focus of this analysis, EPclin was highly prognostic for DR in this sub-group of patients (N=216) (HR=2.41 (1.74-3.35),  $P<0.0001$ ). Numbers of DR in nodal subgroups were too small to make a meaningful interpretation (data not shown).

## Discussion

This combined analysis of ABCSG-6, ABCSG-8 and TransATAC comprises the biomarker cohorts from three similar prospective phase III randomised trials that included postmenopausal patients with ER-positive, HER2-negative early breast cancer. All of these patients were treated with endocrine therapy (Anastrozole, Tamoxifen alone or sequentially) after completion of local therapy and in the absence of chemotherapy. This retrospective analysis of prospectively recorded long-term follow-up data includes a total of 2630 patients, of which 470 women had been diagnosed with a lobular breast cancer subtype. This cohort provides the opportunity to apply the validated gene expression signature EPclin to the largest, to our knowledge, lobular breast cancer cohort from phase III randomized trials.

Our results show that in this cohort of mostly low to intermediate risk patients, EPclin categorised almost two thirds (63%) of ILC cases into low risk, with 10-year DR risks of 4.8%. These results suggest a very favourable outcome, especially in comparison to women in the high-risk category, which had a 10-year DR risk of 26.6%. This subgroup of high risk patients, which comprises 30% of all women with ILC, creates a strong rationale for further studies to evaluate chemopredictive ability by EndoPredict. The absolute risk difference between low and high risk patients with ILC was 21.8%. The prognostic value of the test was similar for patients with and without lymph node metastasis and for late recurrences (late distant metastasis after five years of follow-up). Importantly, our results showed that in all subgroups and for early and late DR, EPclin provided substantially more prognostic information for ILCs than clinicopathological parameters as captured by the CTS alone.

In the prognostic comparison of EPclin between lobular and ductal breast cancer no significant differences were observed, but numerically a higher relative number of lobular breast cancers seemed to be categorized into the low-risk group and the average 10-year risk of distant recurrence appeared higher for lobular cancers in the molecular high-risk group. The performance was surprisingly similar in ILC compared to IDC cancer despite clinical evidence attesting to biologic differences between the subtypes. Several lines of clinical evidence suggest that the term lobular breast cancer encompasses a distinct clinical entity. Lobular morphology is associated with distinct radiological features such as multifocality and extensive intraductal components, distinct pathological features such as growth in sheets and lack of E-cadherin expression [19]. The propensity for low-volume lymph node involvement is a particular challenge for clinical decision making [20]. Reports suggest that there is a 24% discordance in cases of low-volume nodal metastasis [21] and better definitions in guidelines need to be defined. Moreover, response to neoadjuvant chemotherapy is poor in women with ILC (pCR 4.2% in hormone receptor positive breast cancer)

[22]. In terms of local therapy, these features and diffuse nature of ILCs lead to higher rates of second surgeries and higher mastectomy rates [23].

Genomic characterization shows two thirds of cases with lobular morphology harbouring mutations in *CDH1* and half of lobular cases with alterations in *PIK3CA*, *PTEN* and *AKT1* [24]. Activation of the the human epidermal growth factor receptor pathway, via HER2 and HER3 mutations, show higher frequency in lobular as compared to ductal invasive breast cancer. The genomic alterations found in lobular breast cancer are often quantitatively, but in several instances, qualitatively distinct from invasive ductal morphologies [25]. Therefore, the successful validation of EPclin in this subtype was an important but not entirely expected finding. It is tempting to speculate that a gene expression test that comprises anatomical information, proliferation and ER signalling/differentiation genes may be sufficient to improve the prognostication of patients with ILC. For truly predictive information concerning benefit from systemic adjuvant therapies, a detailed and reliable detection of DNA alterations may be necessary.

Our study has strengths and limitations. Strengths include the inclusion of a large cohort of well characterised women with histologically confirmed ILC. Furthermore, we have clinical outcome data for all patients and used well characterised tissue samples. EPclin for all trials was measured in the same laboratory, with all personnel being blinded to clinical outcome data. Limitations include small sub-groups of patients with node-positive disease and a proportion of women with ILC for whom local grading was not determined. In this analysis, we were unable to determine the chemopredictive value of EPclin in women with ILC. Our results are only applicable for women who have received endocrine therapy only and cannot be extrapolated to those who have received chemotherapy.

In summary, EPclin showed excellent prognostic performance in ILC, for both lymph node negative and lymph node positive disease and for both early and late time points of distant recurrence. For the clinician, the added information of EPclin in this subset of patients may be of particular value: lobular breast cancer can often reach large total tumour diameters due to the sheet-like pattern of growth. Nodal metastasis can be frequent and of low volume, and the typical anatomical information may therefore appear less reliable. In addition, the described poor tumour shrinkage from neoadjuvant chemotherapy make clinical decisions concerning adjuvant cytotoxic treatment daunting at times. In this respect, EPclin is of added prognostic value above and beyond clinicopathological assessment in both IDC and ILC may help patients to make better informed decisions concerning adjuvant cytotoxic and/or extended endocrine treatment.

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## Figure Legend

**Figure 1:** Kaplan-Meier graphs and distant recurrence risks (%) according to EPclin risk groups for A) all women with ILC, B) node-negative ILC, and C) node-positive ILC.

**Figure 2:** Forest plot showing HRs with associated 95% CIs for ILC and IDC for overall population and subgroups.

**Table 1:** Baseline characteristics according to histological subtypes.

	<b>ILC (N=470)</b>	<b>IDC (N=1944)</b>	<b>P-VALUE</b>
Age (years), median (IQR)	63 (58-70)	64 (58-71)	0.40
<b>Tumour stage</b>			
T1a/b	49 (10.4%)	346 (17.8%)	
T1c	209 (44.5%)	1020 (52.5%)	
T2	192 (40.9%)	559 (28.8%)	
T3	18 (3.8%)	18 (0.9%)	
Unknown	2 (0.4%)	1 (0.05%)	<0.001
<b>Nodal status</b>			
Negative	326 (69.4%)	1360 (70.0%)	
1-3 positive	115 (24.5%)	490 (25.2%)	
4-10 positive	24 (5.1%)	78 (4.0%)	
10+ positive	5 (1.1%)	16 (0.8%)	0.69
<b>Tumour grade</b>			
Well	49 (10.4%)	482 (24.8%)	
Intermediate	301 (64.0%)	1278 (65.7%)	
Poor	16 (3.4%)	180 (9.3%)	
Undetermined	104 (22.1%)	4 (0.2%)	<0.001
EPclin, median (IQR)	3.07 (2.51-3.55)	3.08 (2.56-3.73)	0.13
CTS, median (IQR)	120.2 (89.9-171.4)	102.0 (64.1-143.6)	<0.001

Legend: For continuous variables the t-test was used and for categorical variables the chi-square test was used to assess differences in baseline characteristics

**Table 2:** Prognostic value of EPclin and CTS for women with ILC.

	HR (95% CI)	P-value	LR- $\chi^2$	$\Delta$ LR- $\chi^2$ (CTS+EPclin vs. CTS)
<b>All</b>				
EPclin (N=470)	3.32 (2.54-4.34)	<0.0001	70.42	17.60
CTS (N=364)	2.17 (1.73-2.72)	<0.0001	36.37	(P<0.0001)
<b>Node-negative</b>				
EPclin (N=326)	2.56 (1.63-4.02)	<0.0001	13.65	8.52
CTS (N=258)	1.28 (0.62-2.64)	0.5	0.44	(P=0.035)
<b>Node-positive</b>				
EPclin (N=144)	3.70 (2.49-5.50)	<0.0001	35.71	9.07
CTS (N=106)	2.12 (1.53-2.93)	<0.0001	18.96	(P=0.0026)
<b>Years 5-10</b>				
EPclin (N=397)	3.83 (2.43-6.04)	<0.0001	32.43	21.38
CTS (N=313)	1.66 (1.12-2.47)	0.012	5.33	(P<0.0001)

Legend: Table shows prognostic value of EPclin according to patient population and time period. The LR test shows how strong a model is, the greater the value the more prognostic value is provided. HR=Hazard Ratio, CI=Confidence Interval, LR=Likelihood Ratio

Figure 1: Kaplan-Meier graphs and distant recurrence risks (%) according to EPclin risk groups for A) all women with ILC, B) node-negative ILC, and C) node-positive ILC.

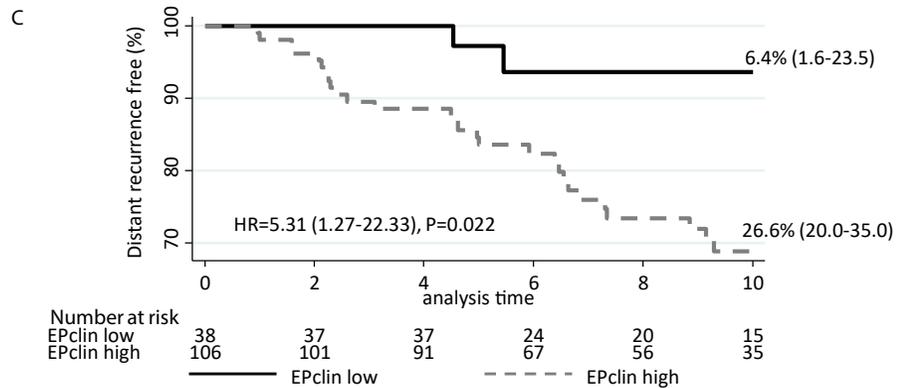
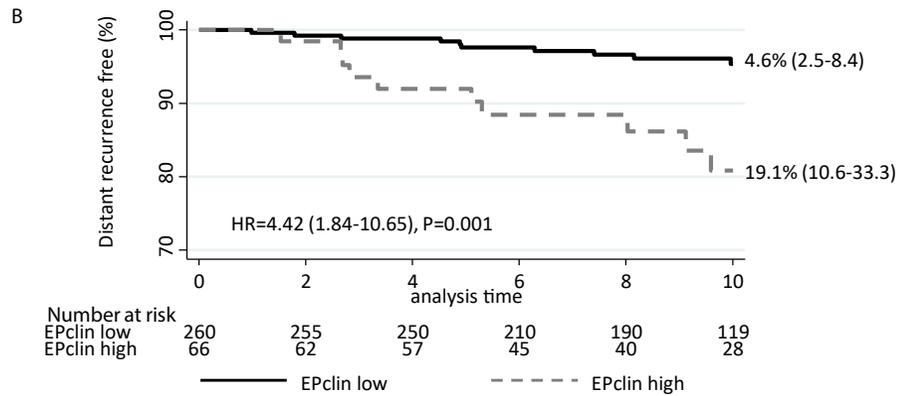
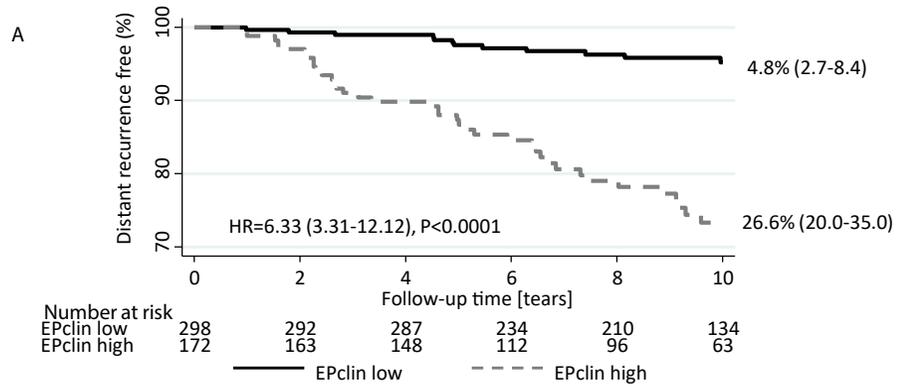
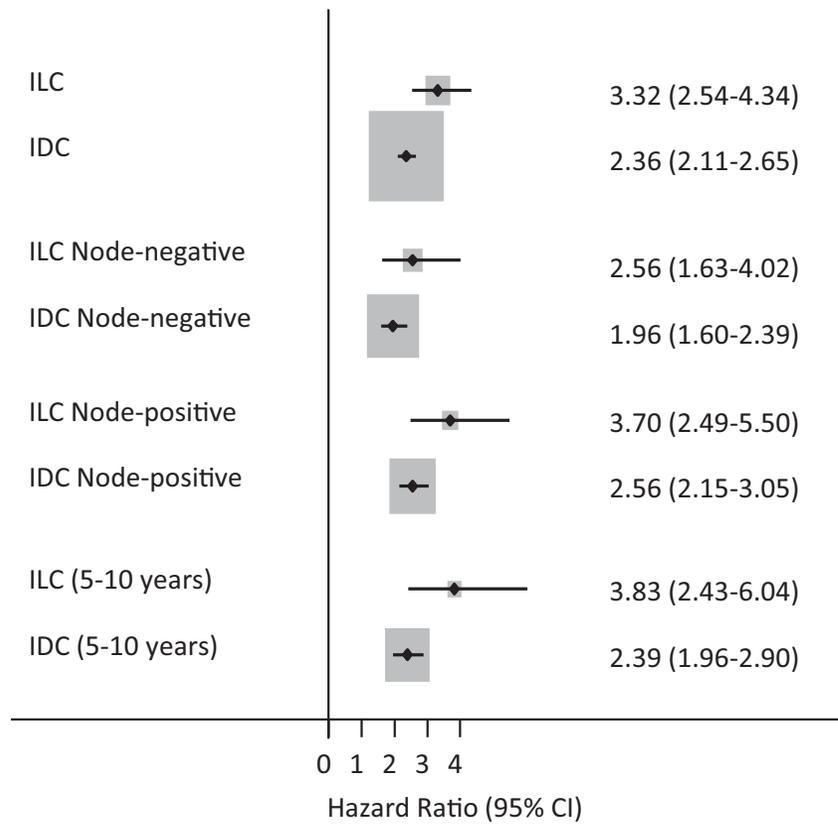


Figure 2: Forest plot showing HRs with associated 95% CIs for ILC and IDC for overall population and subgroups.



# Clinical Cancer Research

## Prognostic Value of EndoPredict in Women with Hormone Receptor Positive, HER2-Negative Invasive Lobular Breast Cancer

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