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Borderline Estrogen Receptor–Positive Breast Cancers in Black and White Women

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Abstract

Background: Some breast tumors expressing greater than 1% and less than 10% estrogen receptor (ER) positivity (ER-borderline) are clinically aggressive; others exhibit luminal biology. Prior ER-borderline studies included few black participants.

Methods: Using the Carolina Breast Cancer Study (phase I: 1993–1996; 2: 1996–2001; 3: 2008–2013), a population-based study that oversampled black women, we compared ER-borderline ($n = 217$) to ER-positive ($n = 1885$) and ER-negative ($n = 757$) tumors. PAM50 subtype and risk of recurrence score (ROR-PT, incorporates subtype, proliferation, tumor size) were measured. Relative frequency differences (RFD) were estimated using multivariable linear regression. Disease-free interval (DFI) was evaluated by ER category and endocrine therapy receipt, overall and by race, using Kaplan Meier and Cox models. Statistical tests were two-sided.

Results: ER-borderlines were more frequently basal-like (RFD = +37.7%, 95% confidence interval [CI] = 27.1% to 48.4%) and high ROR-PT (RFD = +52.4%, 95% CI = 36.8% to 68.0%) relative to ER-positives. Having a high ROR-PT ER-borderline tumor was statistically significantly associated with black race (RFD = +26.2%, 95% CI = 9.0% to 43.3%). Compared to ER-positives, DFI of ER-borderlines treated with endocrine therapy was poorer but not statistically significantly different (hazard ratio [HR] = 2.03, 95% CI = 0.89% to 4.65%), whereas DFI was statistically significantly worse for ER-borderlines without endocrine therapy (HR = 3.33, 95% CI = 1.84% to 6.02%). However, black women with ER-borderline had worse DFI compared to ER-positives, even when treated with endocrine therapy (HR = 2.77, 95% CI = 1.09% to 7.04%).

Conclusions: ER-borderline tumors were genomically heterogeneous, with survival outcomes that differed by endocrine therapy receipt and race. Black race predicted high-risk ER-borderlines and may be associated with poorer endocrine therapy response.

Estrogen receptor (ER) positivity is a prognostic indicator and a powerful predictor of endocrine therapy response in breast cancer (1–4). ER status is typically assessed using immunohistochemical (IHC) stains from which percentage and intensity of positively stained tumor cells can be quantified. In most tumors, ER is either entirely absent or clearly expressed (5,6). However, a small subset of breast tumors exhibits weak

(≥ 1 to $< 10\%$) ER-positivity. Many of these so-called borderline tumors have pathological features of ER-negative tumors, with survival outcomes intermediate between ER-negative and ER-positive cases (7–15). As a result, identifying ER-borderline tumors that are responsive to endocrine therapy has been a persistent clinical challenge. In 2010, the American Society of Clinical Oncology and College of American Pathologists issued

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clinical guidelines classifying ER-positive breast cancers as those with greater than or equal to 1% staining; in these tumors, endocrine therapy is recommended (16). Prior to these guidelines, many clinicians and investigators used greater than or equal to 10% ER positivity to determine eligibility for endocrine therapy (17–19). The evidence supporting the 2010 change in ER threshold was largely derived from clinical trials with low representation of minority women (3,18,19).

The Carolina Breast Cancer Study (CBCS) phase III enrolled black and white women with invasive breast cancer from 2008 to 2013, spanning the 2010 change in clinical guidelines for ER-positivity. This allows a unique opportunity to evaluate ER-borderline outcomes comparing women with guideline-concordant care excluding and including endocrine therapy. Moreover, the study is well positioned to evaluate the role of both race and genomics in ER-borderline breast cancer. The study oversampled black women and conducted genomic profiling to measure intrinsic subtype and risk of recurrence scores.

In this study, we compared the genomic features of ER-borderline tumors to ER-positive and ER-negative tumors in phases I, II, and III of the CBCS and analyzed the disease-free interval (DFI) of women enrolled in phase III to evaluate outcomes of ER-borderline tumors treated with and without endocrine therapy. This work identifies demographic and clinical characteristics associated with poor-prognosis molecular features and assesses whether ER-borderline recurrence rates differ by race or initiation of endocrine therapy.

Methods

Study Population

The CBCS is a population-based study conducted in North Carolina (phase I: 1993–1996; phase II: 1996–2001; and phase III: 2008–2013); study details have been described previously (20,21). [Supplementary Figure 1](#) (available online) depicts study population inclusion. Briefly, women age 20–74 years diagnosed with a first primary invasive breast cancer were enrolled using rapid case ascertainment, oversampling black and younger women (age <50 years). Health history was collected during in-home interviews (22,23). Race was self-reported and categorized as white or black. Less than 2% of nonblack participants self-identified as multiracial, Hispanic, or other race and/or ethnicities and were grouped with white case patients for statistical analyses. The study was approved by the Office of Human Research Ethics at the University of North Carolina at Chapel Hill (UNC). Informed consent was obtained from each participant.

Tumor Characteristics

Tumor size, node status, and stage were abstracted from medical records and pathology reports. Combined grade was centrally assigned by a single breast cancer pathologist (JG) using the Nottingham breast cancer grading system (24); grade was missing for phase II. Progesterone receptor (PR) status was determined from medical records in 80% of cases from phases I and II, and from IHC staining at UNC for remaining cases. HER2 status was determined by IHC staining at UNC for phases I and II and pathology reports for phase III (23).

ER Categories

Quantitative ER data were available for 2859 cases. ER expression was abstracted from medical and pathology records for 496

cases (17%) from phases I and II. Quality assurance studies in CBCS found high accuracy in clinical data ascertainment (23). For remaining cases, formalin-fixed paraffin-embedded (FFPE) tumor tissue blocks were previously sectioned and IHC stained at the Translational Pathology Laboratory at UNC (25). For 1685 cases, multiple tissue microarray cores per case were assessed using automated algorithms (Aperio Technologies, Vista, CA). A core-to-case collapsing method was applied to determine ER percent positivity using a tumor cellularity-weighted approach. Agreement with clinical record was 93% (25). For 679 cases, whole slides were assessed by automated algorithm. Agreement with clinical record was 81% for phases I and II and found to be 89% for phase III (23). For all cases, ER category was defined by percentage of cells staining positively for ER at any intensity, with less than 1% negative, greater than or equal to 1% to less than 10%, and greater than or equal to 10% positive.

Genomic Assessment

The details of RNA isolation and quantification have been published previously (26). Briefly, at UNC central laboratory, RNA was isolated from FFPE tumor blocks using Qiagen FFPE RNeasy kit (Germantown, MD, USA) and quantified using NanoString assay (27). Data that passed quality control using NanoString nSolver software (Seattle, WA, USA) were normalized following nCounter protocol, including background subtraction, positive control normalization, and reference gene normalization. Normalized data were log₂ transformed, standardized across samples, and median centered across genes. Genomic analysis was performed for all participants with tumor blocks available. Relative to participants without genomic data, those with genomic data had larger tumors, higher tumor stage, and higher grade and were more likely to be lymph node positive. There was no statistically significant difference in age at diagnosis.

P53 status was assessed using a previously validated 52-gene TP53-dependent signature (28). Mutant-like vs normal-like class was determined by similarity-to-centroid approach using distance-weighted discrimination (29). ESR1 expression was measured using an ESR1-specific probe and NanoString RNA counting methods.

As described previously, PAM50 predictor was used to categorize tumors as luminal A, luminal B, HER2-enriched, basal-like, and normal-like and to calculate risk of recurrence score (ROR-PT), which incorporates subtype with additional weighting by a proliferation gene signature and tumor size (26,30). The ROR-PT score predicts individual risk of distant recurrence (31,32). Normal-like samples were excluded because of insufficient tumor cellularity (n = 50).

Breast Cancer Recurrence

Recurrence data were available only for phase III (n = 2157). DFI was defined as time from diagnosis to subsequent recurrent breast cancer (local, regional, or distant) (33). Breast cancer recurrence, including date, was verified using the medical records of women who reported having a recurrence during telephone follow-up contact, which occurred at 9, 18, 38, 66, 80, 92, and 104 months from enrollment. Recurrence data in this analysis are complete through September 2018. We excluded women with stage IV disease at first diagnosis (n = 65), ER-positive women who did not receive endocrine therapy (n = 100), and ER-negative women who received endocrine therapy (n = 56). HER2-positive (n = 297) and missing HER2 status (n = 2) were

Table 1. Demographic and clinical characteristics of case patients in Carolina Breast Cancer Study phases I, II, and III by immunohistochemical-defined estrogen receptor category

Characteristic	ER expression		
	<1% (n = 757; 26.5%)	≥1–<10% (n = 217; 7.6%)	≥10% (n = 1885; 65.9%)
Study phase, No. (%)			
1	66 (8.7)	60 (27.7)	133 (7.1)
2	150 (19.8)	17 (7.8)	276 (14.6)
3	541 (71.5)	140 (64.5)	1476 (78.3)
Age at diagnosis, No. (%), y			
<50 (referent)	432 (57.1)	127 (58.5)	857 (45.5)
≥50	325 (42.9)	90 (41.5)	1028 (54.5)
RFD* (95% CI), %	−9.6 (−12.9 to −6.3)	−4.9 (−7.7 to −2.3)	Referent
P†	<.001	<.001	
Menopausal status, No. (%)			
Premenopausal (referent)	373 (49.3)	115 (53.0)	785 (41.6)
Postmenopausal	384 (50.7)	102 (47.0)	1100 (58.4)
RFD* (95% CI), %	−6.5 (−9.9 to −3.2)	−4.3 (−6.9 to −1.6)	Referent
P†	<.001	.002	
Race, No. (%)			
White (referent)	288 (38.0)	118 (54.4)	1103 (58.5)
AA/Black	469 (62.0)	99 (46.6)	782 (41.5)
RFD* (95% CI), %	16.1 (12.6 to 19.4)	1.4 (−1.1 to 3.9)	Referent
P†	<.001	.26	
PR status, No. (%)‡			
Positive (referent)	86 (11)	96 (44)	1613 (86)
Negative	665 (89)	121 (56)	264 (14)
RFD* (95% CI), %	65.7 (62.5 to 68.9)	25.6 (20.8 to 30.4)	Referent
P†	<.001	<.001	
HER2 status, No. (%)§			
Negative	595 (78.7)	141 (65.0)	1427 (75.8)
Positive	123 (16.3)	47 (21.7)	273 (14.5)
Missing	38 (5.0)	29 (13.3)	183 (9.7)
Combined grade, No. (%)			
1	13 (2.2)	30 (18.1)	448 (29.7)
2	84 (14.4)	45 (27.1)	711 (47.2)
3	485 (83.3)	91 (54.8)	349 (23.1)
RFD, grade 3 vs grade 1/2* (95% CI), %	48.6 (44.8 to 52.5)	13.6 (9.5 to 17.7)	Referent
P†	<.001	<.001	
Tumor size, No. (%), cm			
≤2	318 (42.8)	99 (46.7)	1130 (60.7)
>2–≤5	337 (45.4)	93 (43.9)	583 (31.3)
>5	88 (11.8)	20 (9.4)	149 (8.0)
RFD, >2 vs ≤2 (95% CI), %*	5.7 (−0.7 to 12.2)	0.0 (−5.1 to 5.1)	Referent
P†	.08	.99	
Node status, No. (%)			
Negative (referent)	448 (59.4)	134 (62.0)	1187 (63.2)
Positive	306 (40.6)	82 (38.0)	691 (36.8)
RFD* (95% CI), %	0.8 (−2.6 to 4.2)	−0.3 (−2.9 to 2.3)	Referent
P†	.64	.82	
Stage, No. (%)			
I	232 (31.0)	88 (41.3)	904 (48.5)
II	386 (52.5)	85 (39.9)	712 (38.2)
III	112 (15.0)	34 (16.0)	198 (10.6)
IV	19 (2.5)	6 (2.8)	49 (2.6)
RFD, III/IV vs I/II* (95% CI), %	4.9 (0 to 9.9)	3.3 (−0.9 to 7.4)	Referent
P†	.05	.13	

*Relative frequency differences (RFD) adjusted for age and race (except for race models, which were adjusted for age only, and age and menopausal status models, which were adjusted for race only). AA = African American; CI = confidence interval; ER = estrogen receptor; PR = progesterone receptor.

†Two-sided χ^2 test.

‡Fourteen case patients had missing PR status.

§RFD not calculated because of uneven missingness across ER category.

||A total of 603 case patients had missing grade, 42 had missing size, 11 had missing node status, and 34 had missing stage.

excluded. ER-borderlines were grouped by endocrine therapy receipt ($n=36$ received endocrine therapy, $n=58$ did not). Chemotherapy receipt was assessed. We performed a sensitivity analyses excluding 30 women who experienced a recurrence within 1 year of their first primary breast cancer diagnosis.

Statistical Analyses

Linear binomial regression was used to calculate relative frequency differences (RFD), interpretable as the percentage difference between index and referent groups, and 95% confidence intervals (CI). P values were from two-sided χ^2 tests ($\alpha=0.05$).

Finite mixture modeling was used to dichotomize tumors as ESR1 high and ESR1 low (34,35). Using the expectation-maximization algorithm described by Do and Batzoglou (36), we estimated parameters that minimized model deviance and assigned a cutoff as the value at which the probability of belonging to the low-ESR1 peak was equivalent to a type-I error at 0.05. Cutoff selection was performed using R version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria). Median ESR1 expression levels were compared using Kruskal-Wallis test.

We used multiple linear regression to test whether receipt of endocrine therapy among ER-borderlines was associated with clinical variables. Kaplan-Meier curves of DFI were compared using log-rank test with Bonferroni correction for multiple comparisons. P values comparing 5-year recurrence rates were calculated using z-test, assuming normal distribution using the difference of the two rates and standard errors (37). Multivariable Cox proportional hazards models were used to estimate associations between DFI (outcome) and four categories of exposure: ER-positive, ER-borderline with endocrine therapy, ER-borderline without endocrine therapy, and ER-negative. Model 1 adjusted for age and stage. Model 2 additionally adjusted for chemotherapy receipt. Proportional hazards assumptions were assessed visually and found to be valid. The addition of PR status did not substantially change effect estimates (data not shown). We tested for statistical interaction between race and exposure category using likelihood ratio test ($\alpha=0.10$). Statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc, Cary, NC).

Results

Clinical, Demographic, and Genomic Features of ER-Borderline Tumors

We first examined clinical and demographic features by ER category in all three phases of CBCS. The 2859 eligible participants included 1509 (52.8%) white and 1350 (47.2%) black women; 7.6% ($n=217$) of case patients were ER-borderline, 26.5% ($n=757$) were ER-negative, and 65.9% ($n=1885$) were ER-positive. ER-negatives and ER-borderlines both exhibited a statistically significantly younger age at diagnosis and were more common among premenopausal women compared to ER-positives. Frequency of ER-borderline tumors did not vary by race. ER-negatives and ER-borderlines both were more frequently high grade (Table 1).

We next analyzed gene expression profiles of tumors from phases I to III to compare molecular features by ER category. Genomic data were available for 1508 case patients. Intrinsic subtype distribution among ER-borderlines was mixed. ER-negatives and ER-borderlines had a statistically significantly

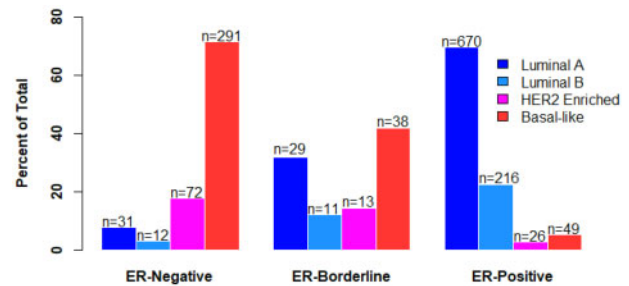


Figure 1. Intrinsic subtype distribution by estrogen receptor (ER) category in Carolina Breast Cancer Study phases I, II, and III. ER-negatives and ER-borderlines had a statistically significantly higher frequency of HER2-enriched (17.7% ER-negative, relative frequency difference [RFD] = +67.5%, 95% confidence interval [CI] = 58.6 to 76.4, $P < .001$; 14.3% ER-borderline, RFD = +27.2%, 95% CI = 12.2 to 42.2, $P < .001$; two-sided χ^2 test) and basal-like subtypes (71.7% ER-negative, RFD = +80.3%, 95% CI = 76.1 to 84.4, $P < .001$; 41.8% ER-borderline, RFD = +37.7%, 95% CI = 27.1 to 48.4, $P < .001$; two-sided χ^2 test) relative to ER-positive (2.7% HER2-enriched, 5.1% basal-like).

higher frequency of HER2-enriched (17.7% ER-negative, RFD = +67.5%, 95% CI = 58.6 to 76.4; $P < .001$; 14.3% ER-borderline, RFD = +27.2%, 95% CI = 12.2 to 42.2; $P < .001$; two-sided χ^2 test) and basal-like subtypes (71.7% ER-negative, RFD = +80.3%, 95% CI = 76.1 to 84.4; $P < .001$; 41.8% ER-borderline, RFD = +37.7%, 95% CI = 27.1 to 48.4; $P < .001$; two-sided χ^2 test) relative to ER-positive (2.7% HER2-enriched, 5.1% basal-like) (Figure 1). The relative frequency of high ROR-PT score was statistically significantly higher both for ER-negatives (RFD = 67.9%, 95% CI = 61.9 to 73.8; $P < .001$) and ER-borderlines (RFD = +52.4%, 95% CI = 36.8 to 68.0; $P < .001$) vs ER-positives (Table 2).

ER-borderlines also varied with respect to p53 status and quantitative ESR1. ER-negative and ER-borderline tumors both were more likely to be p53 mutant-like and ESR1-low than ER-positive tumors. Median ESR1 expression level of ER-borderline was statistically significantly different from ER-negative ($P < .001$, Kruskal-Wallis test) and ER-positive ($P < .001$, Kruskal-Wallis test) with highest expression among ER-positives (median = 10.3, interquartile range [IQR] = 9.3–11.3) followed by ER-borderlines (median = 7.1, IQR = 4.9–9.3) then ER-negatives (median = 5.1, IQR = 4.0–6.6) (Supplementary Figure 2, available online).

We next assessed whether demographic and clinical variables could identify ER-borderline tumors with poor-prognosis genomic features in phases I–III. Black race was statistically significantly associated with high ROR-PT among borderline tumors (38.8% black vs 12.5% white women, RFD = +26.2%, 95% CI = 9.0 to 43.3) (Table 3). High tumor grade was statistically significantly associated with ER-borderlines of basal-like subtype (55% high vs 15% low or medium grade, RFD = +41.9%, 95% CI = 17.7 to 66.0).

ER-Borderline Tumor Association With Recurrence Outcomes

Phase III enrollment spanned 2008–2013 and included women with HER2-negative ER-borderline tumors who received treatment with and without endocrine therapy (16). The recurrence rates for these tumors were compared to those of ER-negatives treated without endocrine therapy and ER-positives treated with endocrine therapy. A total of 189 recurrences occurred over median follow-up of 6.69 years (range = 0.40–9.96). Chemotherapy use was 82.8% ($n=48$) among ER-borderlines without endocrine therapy, 69.4% ($n=25$) among ER-borderlines

Table 2. Distribution of genomic tumor characteristics by immunohistochemical-defined estrogen receptor category in Carolina Breast Cancer Study phases I, II, and III

Characteristic	ER expression		
	<1% (n = 422; 28%)	≥1 to <10% (n = 96; 6%)	≥10% (n = 990; 66%)
ROR-PT*			
Mean score (SD)	60.24 (20.72)	48.61 (21.61)	32.31 (22.30)
Low, No. (%)	19 (4.6)	8 (8.5)	277 (28.3)
Medium, No. (%)	217 (52.0)	62 (66.0)	622 (63.5)
High, No. (%)	181 (43.4)	24 (25.5)	81 (8.3)
RFD, high vs low or medium (95% CI), %†	67.9 (61.9 to 73.8)	52.4 (36.8 to 68.0)	Referent
P‡	<.001	<.001	
p53 status			
WT, No. (%)	155 (36.7)	49 (51.0)	722 (72.9)
Mutant-like, No. (%)	267 (63.3)	47 (49.0)	268 (27.1)
RFD, mutant-like vs WT (95% CI), %†	29.8 (24.7 to 34.8)	8.0 (3.8 to 12.2)	Referent
P‡	<.001	<.001	
ESR1 expression			
ESR1-low, No. (%)§	372 (88.2)	68 (70.8)	158 (15.9)
ESR1-high, No. (%)§	50 (11.9)	28 (29.2)	836 (84.1)
RFD, low vs high (95% CI), %†	63.2 (58.8 to 67.6)	26.3 (20.2 to 32.5)	Referent
P‡	<.001	<.001	

*Fifty case patients had missing PAM50 subtype, 17 case patients had missing ROR-PT. CI = confidence interval; ER = estrogen receptor; RFD = relative frequency difference; ROR-PT = risk of recurrence, proliferation and tumor size weighted; WT = wild type.

†Adjusted for age and race.

‡Two-sided χ^2 test.

§ER-negative defined as ESR1 messenger RNA (mRNA) less than 8.77, ER-positive defined as ESR1 mRNA greater than or equal to 8.77.

Table 3. Associations of patient and clinical characteristics with high-risk genomic features among estrogen receptor–borderline tumors in Carolina Breast Cancer Study phases I, II, and III

Characteristic	Intrinsic subtype				ROR-PT score			
	Nonbasal n = 53	Basal- like n = 38	Relative frequency difference* (95% CI), %	P†	Low/ medium ROR-PT n = 65	High ROR-PT n = 24	Relative frequency difference* (95% CI), %	P†
Race								
White, No. (%)	24 (60.0)	16 (40.0)	Referent	.77	35 (87.5)	5 (12.5)	Referent	.005
Black, No. (%)	29 (56.9)	22 (43.1)	+3.1 (–17.1 to 23.3)		30 (61.2)	19 (38.8)	26.2 (9.0 to 43.3)	
Age, y								
<50, No. (%)	34 (65.4)	18 (34.6)	Referent	.11	37 (72.6)	14 (27.5)	Referent	.79
≥50, No. (%)	19 (48.7)	20 (51.3)	+16.6 (–3.7 to 37.0)		28 (73.7)	10 (26.3)	–1.2 (–17.7 to 15.2)	
Menopausal status								
Premenopausal, No. (%)	31 (66.0)	16 (34.0)	Referent	0.12	33 (71.7)	13 (28.3)	Referent	.57
Postmenopausal	22 (50.0)	22 (50.0)	+20.6 (–10.8 to 51.9)		32 (74.4)	11 (25.6)	–17.8 (–52.2 to 16.7)	
Grade‡								
Low or intermediate, No. (%)	22 (84.6)	4 (15.4)	Referent	<.001	22 (88.0)	3 (12.0)	Referent	.09
High, No. (%)	24 (45.3)	29 (54.7)	+41.9 (17.7 to 66.0)		32 (61.5)	20 (38.5)	+23.8 (–5.8 to 53.5)	

*Adjusted for age and/or race. CI = confidence interval; ER = estrogen receptor; ROR-PT = risk of recurrence, proliferation and tumor size weighted.

†Two-sided χ^2 test.

‡Twelve participants had missing grade.

with endocrine therapy, 91% (n = 348) among ER-negatives, and 47% (n = 548) among ER-positives. Receipt of endocrine therapy among borderlines was statistically significantly associated with PR-positive status, but not with race, age, year of diagnosis, menopausal status, or stage. ER-negatives and ER-borderlines without endocrine therapy had statistically significantly worse DFI relative to ER-positives, but DFI was not statistically significantly different between ER-positives and ER-borderlines who initiated endocrine therapy (Figure 2). Five-year DFI probability was 93.7% (95% CI = 92.2 to 95.1) for ER-positives, 88.2% (95% CI = 77.3 to 99.1; P = .32, compared to ER-positive, z-test) for ER-

borderlines with endocrine therapy, 77.3% for ER-borderlines without endocrine therapy (95% CI = 66.4 to 88.1; P = .002, compared to ER-positive, z-test), and 80.7% for ER-negatives (95% CI = 76.7 to 84.7; P = < .001, compared to ER-positive, z-test). Compared to ER-positives, hazard of recurrence for ER-borderlines with endocrine therapy was poorer but not statistically significantly different (hazard ratio [HR] = 2.03, 95% CI = 0.89 to 4.65), whereas ER-orderlines without endocrine therapy fared statistically significantly worse (HR = 3.33, 95% CI = 1.84 to 6.02) (Table 4).

DFI was also examined cross-classified on ER category and race, with results suggesting possible interaction between the

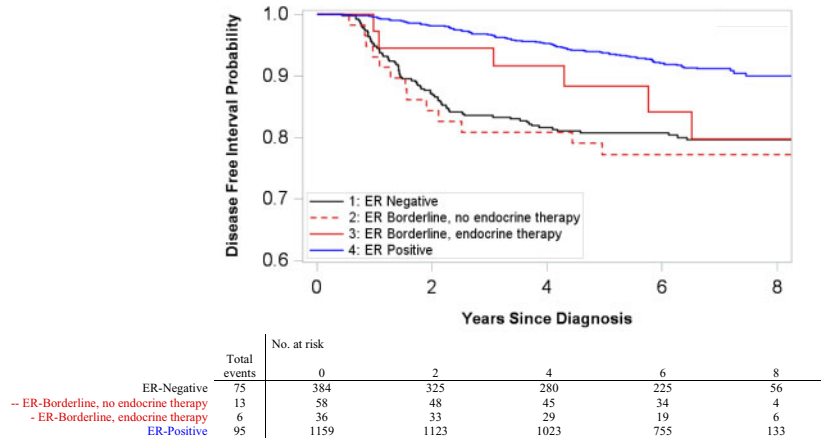


Figure 2. Kaplan-Meier disease-free interval (DFI) curves among HER2-negative case patients by estrogen receptor (ER) category in Carolina Breast Cancer Study phase III. Included ER-positive case patients who received endocrine therapy (ET) and ER-negative case patients who did not receive ET. Pairwise two-sided log-rank tests were performed with Bonferroni correction for multiple comparisons. ER-negative and ER-positive case patients had statistically significantly different DFI ($P < .001$). Similarly, ER-borderline case patients not receiving endocrine therapy and ER-positive case patients had statistically significantly different DFI ($P < .001$). All other pairwise comparisons were not statistically significant.

Table 4. Adjusted hazard ratios for disease-free interval among HER2-negative case patients by estrogen receptor category and endocrine therapy receipt in Carolina Breast Cancer Study phase III

Group	No. (No. of recurrences)	All		White		Black	
		Reduced model*	Full model†	Reduced model*	Full model†	Reduced model*	Full model†
ER-positive‡							
HR (95% CI)	1064 (95)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
P§	—	—	—	—	—	—	—
ER-borderline, no endocrine therapy							
HR (95% CI)	45 (13)	3.27 (1.83 to 5.86)	3.33 (1.84 to 6.02)	4.78 (2.03 to 11.29)	4.22 (1.75 to 10.23)	2.29 (1.03 to 5.11)	2.53 (1.13 to 5.70)
P§	—	<.001	<.001	<.001	.001	.04	.02
ER-borderline, endocrine therapy							
HR (95% CI)	30 (6)	2.01 (0.88 to 4.60)	2.03 (0.89 to 4.65)	1.00 (0.14 to 7.29)	0.99 (0.14 to 7.19)	2.55 (1.01 to 6.44)	2.77 (1.09 to 7.04)
P§	—	.10	.09	.99	.99	.048	.03
ER-negative‡							
HR (95% CI)	309 (75)	2.41 (1.77 to 3.28)	2.45 (1.77 to 3.41)	2.63 (1.59 to 4.35)	2.36 (1.39 to 4.02)	2.14 (1.43 to 3.21)	2.42 (1.57 to 3.74)
P§	—	<.001	<.001	<.001	.002	<.001	<.001

*Adjusted for age, stage. CI = confidence interval; ER = estrogen receptor; HR = hazard ratio.

†Adjusted for age, stage, and chemotherapy receipt.

‡Included ER-positive case patients who received endocrine therapy, included ER-negative case patients who did not receive endocrine therapy.

§Two-sided χ^2 test.

two (likelihood ratio test $P = .11$). Among white women, ER-borderlines with endocrine therapy had recurrence risk similar to ER-positives (HR = 0.99, 95% CI = 0.14 to 7.19, adjusted for age, stage, and chemotherapy receipt), whereas ER-borderlines without endocrine therapy had statistically significantly worse DFI (HR = 4.22, 95% CI = 1.75 to 10.23, adjusted for age, stage, and chemotherapy receipt) (Figure 3). Black women with ER-borderline, however, experienced worse DFI regardless of endocrine therapy receipt (Figure 4). Black women with ER-borderline receiving endocrine therapy had a hazard ratio of 2.77 (95% CI = 1.09 to 7.04, adjusted for age, stage, and chemotherapy receipt); black women not receiving endocrine therapy had a hazard ratio of 2.53 (95% CI = 1.13 to 5.70, adjusted for age, stage, and chemotherapy receipt) (Table 4). Sensitivity analysis excluding 30 recurrences that occurred within 1 year of first primary diagnosis did not change the direction or statistical significance of effect estimates.

Discussion

This study evaluated clinical and genomic features of 217 breast tumors classified as greater than or equal to 1% to less than 10% ER-positive in a large, racially diverse population-based cohort. Relative to ER-positive tumors, ER-borderlines shared many demographic and clinical characteristics with ER-negative tumors, including a higher frequency of younger and premenopausal women, high-grade, high ROR-PT score, and p53 mutant-like status. However, genomic analyses revealed that ER-borderline tumors are more heterogeneous with regard to intrinsic subtype than either ER-negative or ER-positive. DFI in ER-borderlines treated with endocrine therapy was intermediate between ER-negatives and ER-positives, whereas DFI in ER-borderlines without endocrine therapy was statistically significantly worse than ER-positives and statistically indistinguishable from ER-negatives. In race-stratified analyses, the benefit of endocrine

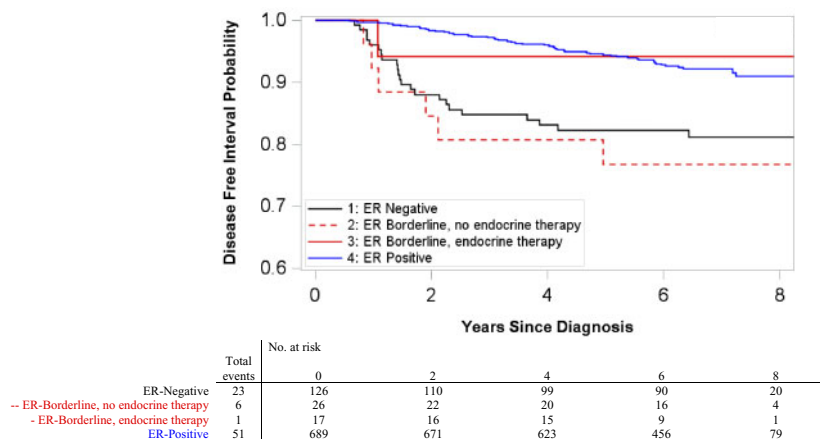


Figure 3. Kaplan-Meier disease-free interval (DFI) curves among HER2-negative white women by estrogen receptor (ER) category in Carolina Breast Cancer Study phase III. Included ER-positive case patients who received endocrine therapy (ET) and ER-negative case patients who did not receive ET. Pairwise two-sided log-rank tests were performed with Bonferroni correction for multiple comparisons. ER-negative and ER-positive case patients had statistically significantly different DFI ($P < .001$). All other pairwise comparisons were not statistically significant.

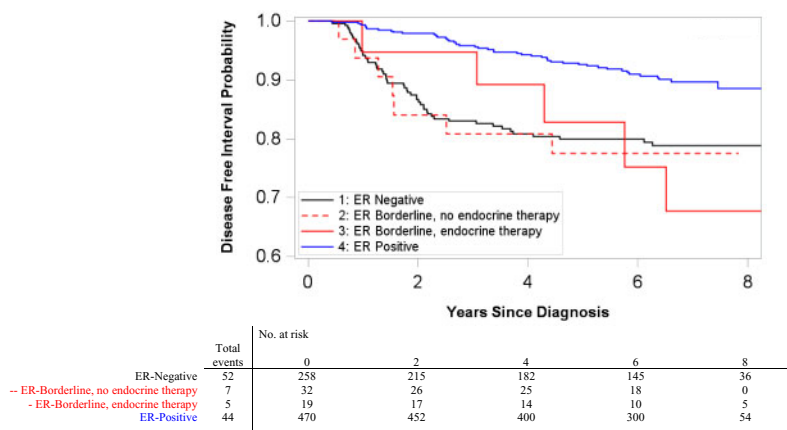


Figure 4. Kaplan-Meier disease-free interval (DFI) curves among HER2-negative black women by estrogen receptor (ER) category in Carolina Breast Cancer Study phase III. Included ER-positive case patients who received endocrine therapy (ET) and ER-negative case patients who did not receive ET. Pairwise two-sided log-rank tests were performed with Bonferroni correction for multiple comparisons. ER-negative and ER-positive case patients had statistically significantly different DFI ($P < .001$). All other pairwise comparisons were not statistically significant.

therapy was evident for white women, but not for black women, who experienced statistically significantly worse DFI when diagnosed with ER-borderline, regardless of endocrine therapy receipt.

Our finding that ER-borderline tumors have genomic characteristics distinct from ER-positives is in line with earlier studies of mostly white women. Multigene RNA-based genomic characteristics have been reported in several cohorts of ER-borderline cases from clinical trials or academic referral centers (7,8,14,15). Our proportion of ER-borderlines, representing only 8% of breast cancers, is consistent with those in earlier reports, which have found the frequency of ER-borderlines to range from 3% to 13%; [Supplementary Table 1](#) (available online) summarizes prior studies for comparison (7-9,14,38,39). The distribution of intrinsic subtypes observed among ER-borderlines resembles a mixture of that seen in the other two ER categories. Here, we report 44% luminal, with the remainder composed of HER2-enriched and basal-like subtype. This closely aligns with Cheang et al. (8), who found 60% of ER 1-9% positive tumors ($n = 65$) were basal-like and HER2-enriched subtype. In contrast, Iwamoto et al. (7)

reported 92% basal-like and HER2-enriched subtype among 1-9% ER-positive cases ($n = 25$), which may be due to the higher proportion of advanced tumors in their study (50% of ER 1-9% were >5 cm in size vs 9% in this study). Iwamoto et al. also found that 1-9% ER tumors had ESR1 expression values similar to ER-negatives, whereas we found that ER-borderline ESR1 expression is statistically distinct both from ER-negative and ER-positive tumors. This suggests a mixed nature of the intrinsic subtype distribution among ER-borderlines.

Because clinical guidelines for ER-positivity changed during the enrollment period of CBCS phase III (2008-2013), our study allowed for analysis of ER-borderline outcomes with and without endocrine therapy initiation. Consistent with prior studies, we found that among all women, ER-borderlines who were treated with endocrine therapy exhibited recurrence rates that were not statistically significantly different from ER-positive tumors, whereas ER-borderlines who did not receive endocrine therapy had recurrence rates similar to ER-negatives and statistically significantly different from ER-positives. This finding may reflect a true association between endocrine therapy and

ER-borderline outcomes, but it is important to recognize that patients in this observational study were not randomly assigned treatment. Patients not receiving endocrine therapy may have been undertreated in other modalities, which were not accounted for in our analysis.

The genomic features and survival outcomes of ER-borderline tumors among black and white women have not been previously examined. Although our race-stratified analyses were limited in precision because of small numbers of participants, the lack of endocrine therapy benefit among black women with ER-borderline tumors may indicate an important disparity. This disparity may reflect differences in tumor biology as indicated by higher ROR-PT scores among ER-borderline black women but may also reflect different rates of endocrine therapy adherence between black and white women. In previous work in CBCS, black women undergoing endocrine therapy experienced a larger burden of side effects and were more likely to report nonadherence than white women (40). Understanding the factors contributing to endocrine therapy nonadherence will be critical to optimizing treatment delivery for all women.

This study has several strengths, including its large cohort of nearly 50% black and white women, enabling a population-based estimate of the prevalence of ER-borderline breast cancer. We also had detailed treatment and follow-up data and centralized genomic analyses. However, these findings are subject to some limitations. First, there are likely factors not accounted for in this analysis that may influence the association between ER-borderline status and risk of recurrence, such as additional treatment modalities, adherence to endocrine therapy, duration of therapy, and time from diagnosis to treatment. Second, our follow-up data are not yet mature enough to include overall or breast cancer-specific survival, or late recurrences. Thus, this analysis reflects patterns within the early window following diagnosis. Finally, ER-borderline is a relatively uncommon breast cancer phenotype. Consequently, although our study is larger than previous studies of ER-borderline tumor genomics, the small number of ER-borderline tumors precluded a more detailed stratification of recurrence by genomic subtype and resulted in low precision in race-stratified analyses. Nonetheless, this study contributes valuable insight to the small subset of breast cancer cases with low percent positivity of ER.

In summary, we found that although many ER-borderline tumors share similarities with ER-negative tumors, these tumors are, as a group, heterogeneous. Black race and high grade at diagnosis predicted high-risk tumor characteristics among ER-borderline cases. Furthermore, we found that whereas white women with ER-borderline who received endocrine therapy had recurrence risk similar to ER-positives, black women with ER-borderline experienced higher recurrence risk regardless of endocrine therapy receipt. Further work is needed to understand how biology and treatment adherence interact to produce disparities in outcomes for black women.

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