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Prognostic and Biologic Significance of ERBB2-Low Expression in Early-Stage Breast Cancer

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IMPORTANCE It is unclear whether ERBB2-low breast cancer should be considered an individual biologic subtype distinct from ERBB2-0 breast cancer.

OBJECTIVE To investigate whether low ERBB2 expression is associated with distinct clinicopathologic characteristics and prognosis among patients with hormone receptor (HR)-positive and triple-negative breast cancer (TNBC).

DESIGN, SETTING, AND PARTICIPANTS This cohort study was conducted using data from a prospectively maintained institutional database on all consecutive patients with breast cancer undergoing surgery between January 2016 and March 2021 at Dana-Farber Brigham Cancer Center. The study included 5235 patients with stage I through III, ERBB2-negative invasive breast cancer. Tumors were classified as ERBB2-low if they had an ERBB2 immunohistochemical (IHC) score of 1+ or 2+ with negative in situ hybridization assay and ERBB2-0 if they had an ERBB2 IHC score of 0. Data were analyzed from September 2021 through January 2022.

EXPOSURES Standard treatment according to institutional guidelines.

MAIN OUTCOMES AND MEASURES Comparison of clinicopathologic characteristics and disease outcomes (pathologic complete response rate [pCR], disease-free survival, distant disease–free survival, and overall survival) between patients with ERBB2-low and ERBB2-0 breast cancer.

RESULTS Among 5235 patients with ERBB2-negative invasive breast cancer (5191 [99.2%] women; median [range] age at primary surgery, 59.0 [21.0-95.0] years), 2917 patients (55.7%) and 2318 patients (44.3%) had ERBB2-low and ERBB2-0 tumors, respectively. Expression of HR was significantly more common among ERBB2-low compared with ERBB2-0 tumors (2643 patients [90.6%] vs 1895 patients [81.8%]; $P < .001$). The rate of ERBB2-low tumors increased progressively, from 296 of 739 estrogen receptor (ER)-negative tumors (40.1%) to 31 of 67 ER-low (ie, ER 1%-9%) tumors (46.3%), 37 of 67 ER-moderate (ie, ER, 10%-49%) tumors (55.2%), 2047 of 3542 ER-high (ie, ER, 50%-95%) tumors (57.8%), and 499 of 803 ER-very high (ie, ER > 95%) tumors (62.1%) ($P < .001$). Among 675 patients receiving neoadjuvant chemotherapy, those with ERBB2-0 tumors experienced higher pCR rates (95 patients [26.8%] vs 53 patients [16.6%]; $P = .002$). However, there were no statistically significant differences in pCR rate between ERBB2-low and ERBB2-0 tumors when separately analyzing HR-positive, ER-low, HR-positive without ER-low, or TNBC tumors. In exploratory survival analysis, no differences by ERBB2-low expression in disease-free survival, distant disease–free survival, or overall survival were observed among patients with HR-positive tumors or TNBC.

CONCLUSIONS AND RELEVANCE The results of this cohort study did not support the interpretation of ERBB2-low breast cancer as a distinct biologic subtype. ERBB2-low expression was positively associated with level of ER expression, and ER-low tumors were enriched among ERBB2-0 tumors, suggesting that, given the worse prognosis of ER-low tumors, they may be associated with confounding of prognostic analyses of ERBB2-low expression.

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Supplemental content

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A wide arsenal of anti-ERBB2 (formerly HER2) drugs has been developed for the treatment of ERBB2-positive breast cancer (BC), allowing for great prognostic improvements for this subtype.[1-3] In contrast, little activity has been observed with most ERBB2-targeting compounds for the approximately 80% of BCs not overexpressing ERBB2,[4-6] currently defined as ERBB2-negative.[7] However, approximately half of these BCs show some degree of ERBB2 immunohistochemical (IHC) expression.[8] These ERBB2-low-expressing tumors[6] were found to respond to novel anti-ERBB2 antibody drug conjugates (ADCs),[9-12] offering the opportunity to expand the targetability of ERBB2 to a much wider population of patients with BC. Two phase 3 trials are currently evaluating the role of trastuzumab deruxtecan in ERBB2-low BC (DESTINY-Breast04 and DESTINY-Breast06), with the DESTINY-Breast04 study reporting positive results.[13]

Nonetheless, relatively little is known about the biology of ERBB2-low BC. This category of tumors is heterogenous, including hormone receptor (HR)–positive BCs and triple-negative BCs (TNBCs).[6,14] Attempts to define clinicopathologic characteristics specific to ERBB2-low BC have yielded inconsistent results.[14-19] While most studies conducted to date have found no prognostic significance associated with ERBB2-low expression,[14-19] few studies have instead suggested a worse[20,21] or better[22,23] prognosis associated with ERBB2-low tumors. For such associations, criticism has been raised regarding the inclusion of estrogen receptor (ER)-low tumors among the HR-positive subgroup, an aspect that may have confounded prognostic analyses.[24]

We analyzed a large cohort of patients identified in a prospectively maintained institutional database of patients with BC undergoing surgery at our academic network. This study aimed to evaluate the biologic and prognostic significance of ERBB2-low expression in BC and to investigate the association between ER and ERBB2-low expression.

Key Points

Question Is ERBB2 (formerly HER2)–low breast cancer a distinct biologic and prognostic subtype?

Findings In this cohort study of 5235 patients with ERBB2-negative invasive breast cancer, most clinicopathologic differences found between ERBB2-low and ERBB2-0 breast cancers were associated with hormone receptor (HR) expression and ERBB2-low expression had no prognostic significance when adjusting for HR status. ERBB2-low and estrogen receptor (ER) expression were found to be positively associated, with most ER-low–expressing tumors being ERBB2-0 and most ER-high–expressing tumors being ERBB2-low.

Meaning These results did not support the interpretation of ERBB2-low as a distinct biologic subtype of breast cancer.

Methods

This cohort study was approved by the Dana-Farber/Harvard Cancer Center institutional review board. Written informed consent was obtained from all patients included in the analysis. We abstracted clinicopathologic data from a prospectively maintained institutional database of consecutive patients with stage I to III BC who underwent surgery at Dana-Farber Brigham Cancer Center from January 2016 to March 2021.

Population

Patients were included if they were diagnosed with histologically confirmed invasive BC, if they had stage I to III disease, and if data regarding ERBB2 IHC score and, when appropriate, ERBB2 in situ hybridization (ISH) status were available. Patients were excluded if their tumor tested ERBB2 positive on presurgical core needle biopsy or surgical specimen. ER and ERBB2 status were abstracted from pathology records. Most patients included in this analysis had surgery performed at Brigham and Women's Hospital (BWH) or Faulkner Hospital and had pathology reviewed by a BWH breast pathologist, except for a minority of patients treated and reviewed at South Shore Hospital (SSH). Tumors were considered HR positive if at least 1% of invasive tumor cells exhibited immunostaining for ER or progesterone receptor (PR). Subset analyses were conducted for patients with low ER expression regardless of PR status and were considered ER low if 1% to 9% of invasive tumor cells exhibited immunostaining for ER or ER positive if at least 10% of invasive tumor cells exhibited immunostaining for ER.

Tumors were considered ERBB2 positive according to the most recent American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines update[7]; these tumors were excluded from our analysis. Per ASCO/CAP guidelines, ERBB2-negative tumors were divided in 2 groups: ERBB2-0 for tumors scored IHC 0 and ERBB2-low for tumors scored IHC 1+ or 2+ with a nonamplified ISH assay.

Clinicopathological Parameters

The following baseline clinicopathological parameters were evaluated by ERBB2-low or ERBB2-0 status: age at primary surgery, sex, race, menopausal status, pathological germline mutation status, clinicopathologic stage, nodal status, tumor histology, tumor grade, ER and PR status, Oncotype DX score, type of breast surgery, axillary surgical management, and chemotherapy and endocrine therapy administration, as well as their specific types. Race was investigator observed, determined by treating physicians for clinical purposes, with the options being African American; Aleutian, American Indian, or Eskimo; Asian or Pacific Islander; White; or unknown. In this study, race was assessed to comprehensively investigate factors potentially associated with ERBB2-low expression. Positive nodal status was derived as clinical and pathological status and determined to be negative if both were found negative and positive if at least 1 was positive. The group with high Oncotype DX risk was defined as including patients with a recurrence score (RS) of 26 or greater, the intermediate-risk group as including patients with an RS between 11 and 25, and the low-risk group as including patients with an RS of 10 or less. Chemotherapy and endocrine therapy were assessed and compared by timing of administration (adjuvant or neoadjuvant) and then evaluated as monotherapy or not.

Statistical Analysis

To compare patient clinicopathologic characteristics, continuous variables were presented as a median with range or a mean with SD and categorical variables were presented as number and percentage. The Wilcoxon rank sum test was used to compare continuous variables, and Fisher exact or χ^2 test were used to compare categorical variables, as appropriate. The association between ordinal ERBB2 expression and ER expression was investigated with the Mantel-Haenszel χ^2 test when ER scores were divided into discrete groups.

Study end points included pathologic complete response (pCR), disease-free survival (DFS), distant DFS (DDFS), and overall survival (OS). We compared pCR rate, defined as ypT0/isN0, between ERBB2-low and ERBB2-0 subgroups. Multivariable analysis for pCR was performed via logistic regression controlling for parameters that were statistically significantly different between the 2 subgroups: menopausal status (postmenopausal, premenopausal, and unknown), tumor grade (grades I-III and unknown), pathogenic germline mutation (yes, no, and not done or unknown), histology (invasive ductal, invasive lobular, mixed, and other) and HR status (positive and negative). DFS was defined as time from the date of primary surgery to the date of disease recurrence or death; patients alive without disease recurrence were censored at the date of last follow-up. DDFS was defined as time from the date of primary surgery to date of the first distant recurrence or death; patients alive without distant recurrence were censored at the date of last follow-up. OS was defined as time from the date of primary surgery to time of death or last follow-up. Disease-free survival, DDFS, and OS were analyzed using univariate Cox proportional hazards models to estimate hazard ratios with 95% CIs. Analyses were conducted using R statistical software version 4.0.3 (R Project for Statistical Computing), and 2-sided *P* values < .05 were considered statistically significant. Data were analyzed from September 2021 through January 2022.

Results

A total of 5235 patients with ERBB2-negative tumors met inclusion criteria and were evaluated in this study (5191 [99.2%] women; median [range] age at primary surgery, 59.0 [21.0-95.0] years), including 4416 patients (84.4%) receiving surgery at BWH or Faulkner Hospital and 819 patients (15.6%) receiving surgery at SSH. There were 228 African American individuals (4.5%); 8 American Indian, Aleutian, or Eskimo individuals (0.2%); 202 Asian or Pacific Islander individuals (4.0%); 4594 White individuals (91.3%); and 203 individuals with unknown race (3.9%). In the overall study population, 2917 patients (55.7%) had ERBB2-low tumors and 2318 patients (44.3%) had ERBB2-0 tumors. Baseline demographics and clinicopathological characteristics by ERBB2 status are summarized in eTable 1 in the Supplement.

Clinicopathologic Characteristics

Hormone receptor expression was significantly more common among ERBB2-low tumors compared with ERBB2-0 tumors (2643 patients [90.6%] vs 1895 patients [81.8%]; *P* < .001); conversely, TNBCs were significantly more prevalent among ERBB2-0 tumors compared with ERBB2-low tumors (423 patients [18.2%] vs 274 patients [9.4%]; *P* < .001). In terms of ERBB2-low rates, 2643 of 4538 HR-positive tumors were ERBB2-low (58.2%), whereas 274 of 697 TNBCs were ERBB2-low tumors (39.3%).

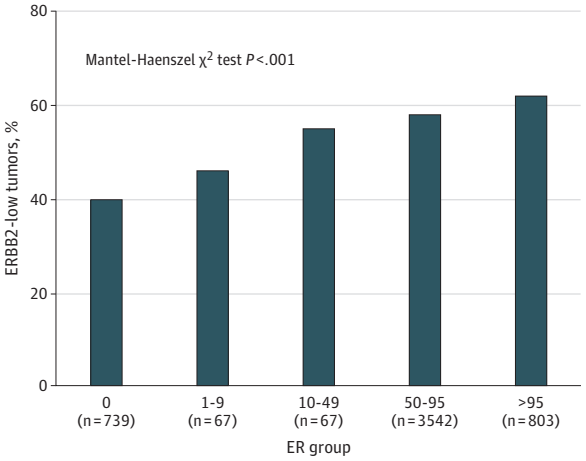
Among patients with ERBB2-low tumors vs those with ERBB2-0 tumors, there were more men with BC (35 men [1.2%] vs 9 men [0.4%]; *P* = .001), more patients who were premenopausal (985 patients [34.5%] vs 720 patients [31.3%]; *P* = .02), fewer high-grade tumors (663 patients [23.0%] vs 691 patients [30.3%]; *P* < .001), and fewer tumors with lobular histology (330 patients [11.3%] vs 338 patients [14.6%]; *P* < .001). Other statistically significant differences between ERBB2-low and ERBB2-0 tumors were observed in frequency of pathogenic germline mutations, frequency of patients tested with Oncotype DX, receipt of any chemotherapy, and receipt of endocrine therapies (eTable 1 in the Supplement). There was no significant difference between the 2 groups in terms of age, race, clinical and pathological stage, nodal status, Oncotype DX RS (median score or risk group distribution), type of breast surgery, or axillary management status.

A multivariable logistic regression (eFigure 1 in the Supplement) was conducted to assess the association between ERBB2 and HR status. After adjustments for clinicopathological characteristics that were significantly unevenly distributed by ERBB2 group, HR remained a factor associated with ERBB2 status (adjusted odds ratio [OR] for HR-positive vs HR-negative tumors, 2.1 [95% CI, 1.73-2.55]; *P* < .001).

Association Between ER and ERBB2 Expression

The distribution of ERBB2-low expression by ER expression is shown in **Figure 1**. When ER expression was divided into dis-

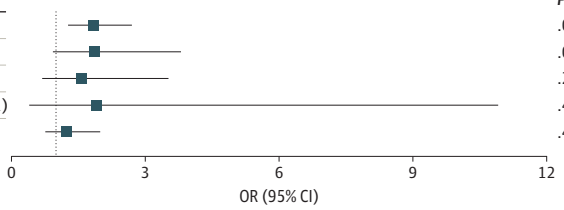
Figure 1. Proportion of ERBB2-Low Tumors by Estrogen Receptor (ER) Expression Threshold



A progressive increase in the proportion of ERBB2-low–expressing tumors was observed with increasing thresholds of ER expression (*P* < .001). Most triple-negative breast cancers and ER-low–expressing tumors were ERBB2-0, whereas most ER-high–expressing tumors were ERBB2-low expressing.

Figure 2. Pathologic Complete Response (pCR) Rate by ERBB2 Expression and Hormone Receptor (HR) Status

Population	No.	pCR, %		OR (95% CI)	P value
		ERBB2-0	ERBB2-low		
All	675	26.8	16.6	1.84 (1.27-2.70)	.002
HR positive	343	14.0	8.0	1.87 (0.93-3.80)	.08
HR positive excluding ER low	313	10.4	6.9	1.56 (0.69-3.52)	.28
ER low	30	38.9	25.0	1.91 (0.40-10.91)	.43
TNBC	332	35.4	30.8	1.23 (0.76-1.99)	.40



In univariate analysis, a significantly higher pCR rate, defined as ypT0/isN0, was found among ERBB2-0 tumors compared with estrogen receptor (ER)-low tumors. However, when analyzing pCR rates in different clinically relevant subgroups, no statistically significant differences in pCR were observed between ERBB2-low and ERBB2-0 tumors among HR-positive, HR-positive excluding ER-low, ER-low, or triple-negative breast cancer (TNBC) tumors.

crete ordinal categories (negative, 0%; low, 1%-9%; moderate, 10%-49%; high, 50%-95%; very high, >95%), there was an association between ERBB2 and ER expression (Mantel-Haenszel χ^2 test $P < .001$), with higher ER expression associated with higher ERBB2 expression. The rate of ERBB2-low tumors increased progressively, from 296 of 739 ER-negative tumors (40.1%) to 31 of 67 ER-low (ie, ER 1%-9%) tumors (46.3%), 37 of 67 ER-moderate (ie, ER 10%-49%) tumors (55.2%), 2047 of 3542 ER-high (ie, ER 50%-95%) tumors (57.8%), and 499 of 803 ER-very high (ie, >95%) tumors (62.1%) ($P < .001$).

Disease Outcomes in ERBB2-Low vs ERBB2-0 Tumors

Among all patients, 675 individuals received neoadjuvant chemotherapy and were evaluable for pathological outcomes, of which 320 patients (47.4%) had ERBB2-low and 355 patients (52.6%) had ERBB2-0 tumors. A significantly higher pCR rate was found among ERBB2-0 tumors compared with ERBB2-low tumors (95 patients [26.8%] vs 53 patients [16.6%]; OR, 1.84 [95% CI, 1.27-2.70]; $P = .002$) (**Figure 2**). However, when analyzing pCR rates in different clinically relevant subgroups, no statistically significant differences in pCR were observed between ERBB2-low and ERBB2-0 tumors among all HR-positive tumors (16 patients [8.0%] vs 20 patients [14.0%]; $P = .08$) or when restricting the analysis to HR-positive excluding ER-low (13 patients [6.9%] vs 13 patients [10.4%]; $P = .28$), ER-low (3 patients [25.0%] vs 7 patients [38.9%]; $P = .43$), or TNBC (37 patients [30.8%] vs 75 patients [35.4%]; $P = .40$) tumors. Moreover, there was no statistically significant difference in pCR rate when adjusting for confounders (menopausal status, HR status, tumor grade, presence of a pathogenic germline mutation, and tumor histology) (eTable 2 in the Supplement).

In an exploratory survival analysis, with approximately 10 months of median follow-up (median [IQR], 9.76 [6.01-40.37] months), significant differences were observed for DFS, DDFS, and OS between ERBB2-low and ERBB2-0 groups. However, there was no significant difference when separately analyzing HR-positive (regardless of Oncotype DX risk category) and TNBC tumors (eFigures 2-3 in the Supplement) or when adjusting for confounders. For example, among all patients, the hazard ratio was 1.13 (95% CI, 0.84-1.52; $P = .40$) for DFS, 1.12 (95% CI, 0.83-1.50; $P = .47$) for DDFS, and 1.14 (95% CI, 0.77-1.67; $P = .52$) for OS (**Table**).

Discussion

This cohort study is one of the largest analyses, to our knowledge, aimed at comprehensively evaluating the prognostic and biologic significance of ERBB2-low expression for BC. Although we found clinicopathologic differences between ERBB2-low and ERBB2-0 tumors, the difference that was largest numerically and most clinically relevant was in HR expression. ERBB2-0 tumors were significantly enriched in TNBC compared with ERBB2-low tumors. The increase in high-grade tumors, germline pathogenic variant carriers, and patients receiving chemotherapy among those with ERBB2-0 tumors was likely associated with this difference. Conversely, there was an increase in men with BC, patients receiving Oncotype DX testing, and patients receiving endocrine treatment among patients with ERBB2-low disease, who were enriched in HR-positive tumors. Overall, most clinicopathologic differences observed may have been associated with the different distribution in HR-positive and TNBC tumors.

Additionally, we found that ER and ERBB2 had a positive association, with the rate of ERBB2-low tumors increasing progressively with increased ER expression. This association may be a confounder in comparisons between ERBB2-low and ERBB2-0 tumors, given that the first are expected to be enriched in luminal-like, high-ER-expressing tumors, whereas the second are more likely to be enriched in basal-like, low-ER-expressing tumors. This is supported by PAM50 analyses, which have found a higher rate of luminal A tumors among ERBB2-low tumors and a higher rate of basal-like tumors among ERBB2-0 tumors.[14]

Consistent with prior reports,[22] we found that ERBB2-low tumors had a lower pCR rate compared with ERBB2-0 tumors (16.6% vs 26.8%; $P = .002$). However, there was no significant difference when restricting the analysis to patients with HR-positive tumors (8.0% vs 14.0%; $P = .08$), and pCR rates were similar when removing patients with ER-low tumors from the HR-positive subgroup (6.9% vs 10.4%; $P = .28$). Similarly, no difference in pCR rate by ERBB2-low expression

was observed among patients with ER-low or TNBC tumors, and both subgroups notably showed high pCR rates, suggesting the similar biology of the 2 entities.[25] Lastly, we found no prognostic significance in terms of survival outcomes for ERBB2-low expression among patients who had TNBC or HR-positive tumors, regardless of Oncotype DX RS score.

The lack of distinctive clinicopathologic features or prognostic value for ERBB2-low expression is not surprising, given that the current definition of ERBB2-low expression imperfectly divides ERBB2-expressing from non–ERBB2-expressing tumors. First, according to the latest guidelines,[7] the ERBB2-0 (IHC 0) category includes tumors that faintly express ERBB2 in 10% or less of tumor cells. The potential relevance of this faint expression is suggested by the inclusion of these tumors in the DESTINY-Breast06 trial,[26] 1 of 2 ongoing confirmatory trials of trastuzumab deruxtecan for ERBB2-low BC. This suggests that the distinction between ERBB2-low and ERBB2-0 BC may be arbitrary and may not truly dissect ERBB2-expressing from non–ERBB2-expressing tumors.

Second, technical aspects related to ERBB2 testing methods may be associated with extensive variability in ERBB2 expression.[6] In a 2022 study[27] aimed at evaluating the scoring accuracy for ERBB2 IHC in the low range (ie, 0 and 1+), there was 26% agreement between pathologists. That study further suggests the inaccuracy of IHC in differentiating ERBB2-low from ERBB2-0 tumors, given that the assay was not optimized for this purpose but rather to identify patients overexpressing ERBB2 and thus expected to benefit from trastuzumab.[28] Importantly, preanalytical and analytical variables in ERBB2 IHC testing may also be associated with the varying rate of ERBB2-low tumors found in multiple studies. Indeed, while our study found that 56% of ERBB2-negative BCs had ERBB2-low expression, other large studies have found rates ranging from 31%[29] to 64%.[16]

Third, the clinical value of distinguishing ERBB2-0 from ERBB2-low expression has been challenged by prospective data showing activity of an anti-ERBB2 ADC in ERBB2-0 BC. Indeed, in the DAISY phase 2 trial, trastuzumab deruxtecan achieved an objective response rate of 30% (11 of 37 patients) for the treatment of metastatic BC scored ERBB2 IHC 0, with a median duration of response of 6.8 months.[30] This finding suggests the inadequacy of IHC in selecting patients for treatment with novel anti-ERBB2 ADCs given that patients with ERBB2-0 tumors may derive a relevant clinical benefit from treatment with trastuzumab deruxtecan. The recent report of positive results from the DESTINY-Breast04 phase 3 trial[13] makes the elucidation of this aspect particularly urgent. Patients were enrolled in the study based on an ERBB2 IHC score of 1+ or 2+ with negative ISH, and this inclusion criteria may affect the drug label in case of approval, despite multiple challenges with IHC testing previously described. Further studies are required to understand if novel quantitative ERBB2 assays may better refine treatment selection for novel ADCs.

Table. Multivariable Analysis of Survival Outcomes

Outcome	ERBB2-0 vs ERBB2-low tumor, hazard ratio (95% CI)[a]	*P* value for log rank
Overall[b]		
DFS (187 events)	1.13 (0.84-1.52)	.40
DDFS (185 events)	1.12 (0.83-1.50)	.47
OS (110 events)	1.14 (0.77-1.67)	.52
HR-positive population[c]		
DFS (117 events)	1.35 (0.94-1.95)	.11
DDFS (116 events)	1.33 (0.92-1.93)	.13
OS (61 events)	1.34 (0.80-2.23)	.26
TNBC population[d]		
DFS (70 events)	0.81 (0.50-1.30)	.38
DDFS (69 events)	0.82 (0.51-1.33)	.43
OS (49 events)	0.88 (0.49-1.56)	.65

Abbreviations: DDFS, distant disease–free survival; DFS, disease-free survival; HR, hormone receptor; OS, overall survival; TNBC, triple-negative breast cancer.

[a] No significant differences in DFS, DDFS, or OS were observed between ERBB2-low and ERBB2-0 tumors after adjusting for confounders.

[b] Adjusted for menopausal status, HR status, tumor grade, pathogenic germline mutation (DFS and DDFS only), and histology.

[c] Adjusted for menopausal status, tumor grade, pathogenic germline mutation (DFS and DDFS only), and histology.

[d] Adjusted for menopausal status, pathogenic germline mutation (DFS and DDFS only), and histology.

Strengths and Limitations

Our study has several strengths and limitations. It was conducted in a clinical setting in a single academic network, making the results exploratory. The study has a large sample size, however, including more than 5000 consecutive patients with nonmetastatic BC receiving surgery at Dana-Farber Brigham Cancer Center. Surgical samples were analyzed at Brigham and Women's Cancer Center by experienced pathologists. Nonetheless, it should be noted that the attribution of ERBB2 IHC scores happened mostly at a time when the distinction between 0 and 1+ scores was not incorporated in clinical management decisions, and no central ERBB2 testing was performed. Although most HR and ERBB2 stains were reviewed by BWH breast pathologists, a minority of stains were reviewed at an outside center (SSH). Additionally, given the prospective nature of the database and its focus on initial diagnosis and treatment of patients, longer-term patient follow-up is limited at this time, warranting caution in the interpretation of survival associations.

Conclusions

The results of this cohort study do not support the interpretation of ERBB2-low tumors as a distinct biologic subtype of BC. Among traditionally ERBB2-negative tumors, ERBB2 and ER expression were positively associated, with most ER-low tumors being ERBB2-0 and most ER-high tumors being ERBB2-low tumors.

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Author Contributions: Dr Tarantino and Ms Jin had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
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