Genomic Characterization and Prognostic Significance of Human Epidermal Growth Factor Receptor 2–Low, Hormone Receptor–Positive, Early Breast Cancers From the BIG 1-98 and SOFT Clinical Trials

Stephen J. Luen, MD, PhD[1,2]; Lauren C. Brown, MD[1,2]; Courtney T. van Geelen, MSc, PhD[2]; Peter Savas, MD, PhD[1,2]; Roswitha Kammler, BA[3]; Patrizia Dell'Orto, ScD[4,5]; Olivia Biasi, DSc[4,5]; Alan S. Coates, MD[6]; Richard D. Gelber, PhD[7]; Beat Thürlimann, MD[8,9]; Marco Colleoni, MD[10]; Gini F. Fleming, MD[11]; Prudence A. Francis, MD[1,2]; Meredith M. Regan, ScD[12]; Giuseppe Viale, MD[4,5]; and Sherene Loi, MD, PhD[1,2]

DOI https://doi.org/10.1200/PO-24-00599

ABSTRACT

PURPOSE To investigate whether hormone receptor–positive, human epidermal growth factor receptor 2–low (HR+HER2-low) versus HR+HER2-zero early breast cancers have distinct genomic and clinical characteristics.

METHODS This study included HR+, HER2-negative early breast cancers from patients enrolled in the phase III, randomized BIG 1-98 and SOFT clinical trials that had undergone tumor genomic sequencing. Tumors were classified HR+HER2-low if they had a centrally reviewed HER2 immunohistochemistry (IHC) score of 1+ or 2+ with negative in situ hybridization and HR+HER2-zero if they had an HER2 IHC score of 0.

RESULTS A total of 1,795 tumors were evaluable for this study (BIG 1-98 n = 520, SOFT n = 1,275). The frequency of HER2-low tumors was 37% and 21% in the postmenopausal BIG 1-98 and premenopausal SOFT cohorts, respectively. There were no significant differences in clinicopathologic variables between HER2-low and HER2-zero groups which was consistent across both trials. There was no significant difference in risk of distant recurrence for patients with HER2-low tumors versus HER2-zero tumors (5-year % distant recurrence-free 94.0% *v* 92.8%, *P* = .61, in BIG 1-98; 89.4% *v* 92.7%, *P* = .31, in SOFT, respectively). Somatic genomic profiles were similar with the exception of *MAP3K1* mutations which were more frequent in HER2-zero tumors (BIG 1-98 19% *v* 5%, SOFT 11% *v* 6%). Both *ERBB2* copy number and *ERBB2* gene expression abundance were significantly higher in HER2-low tumors compared with HER2-zero tumors; however, the absolute difference was small. Correlation between *ERBB2* copy number values and gene expression was modest (*r* = 0.17).

CONCLUSION In two large clinical trials with centrally reviewed HER2 IHC, our findings do not support HER2-low breast cancer as a distinct clinical or biologic entity among HR+HER2- early breast cancers. Absolute differences in median *ERBB2* copy number levels or gene expression are small and of unclear biologic relevance.

ACCOMPANYING CONTENT

Data Sharing Statement

Data Supplement

Accepted December 18, 2024
Published January 24, 2025

JCO Precis Oncol 9:e2400599
© 2025 by American Society of Clinical Oncology

INTRODUCTION

Human epidermal growth factor receptor 2 (HER2)–low breast cancer is a recently described entity defined as tumors with a HER2 immunohistochemistry (IHC) score of 1+ or 2+ and nonamplified on in situ hybridization (ISH).[1] This definition was initially used for clinical trial patient selection and is now established in clinical practice after demonstration of significant clinical benefit from the anti-HER2 antibody-drug conjugate (ADC), trastuzumab deruxtecan compared with standard chemotherapy in previously treated advanced breast cancer, leading to US Food and Drug Administration approval.[2] This is a significant development in HER2-directed therapeutics because it lowers the ERBB2 expression threshold for prediction of benefit, where adjuvant trastuzumab has previously failed.[3] Furthermore, a significant proportion of breast cancers are HER2-low ranging from approximately 40% in triple-negative breast

CONTEXT

Key Objective

To determine whether hormone receptor–positive, human epidermal growth factor receptor 2–low (HR+HER2-low) breast cancers have distinct clinical or genomic differences compared with HR+HER2-zero breast cancers.

Knowledge Generated

Using ERBB2 immunohistochemistry (IHC) expression and tumor genomic data from 1,795 patients from the randomized, phase III, premenopausal SOFT and postmenopausal BIG 1-98 clinical trials, we found no significant differences in clinical and genomic driver alterations between HR+HER2-low breast cancers compared with HR+HER2-zero breast cancers. These data do not support HER2-low breast cancer as a distinct biologic entity among HR+ breast cancers.

Relevance

Among HR+ breast cancers, HER2-low status as determined by IHC (HER2-low *v* HER2-zero) is currently a biomarker to determine eligibility for HER2-directed antibody-drug conjugate therapy (trastuzumab deruxtecan); however, it does not define distinct clinical or biologic entities.

cancers to approximately 60% in HR+ breast cancers.[4,5] Our research focuses on the genomic and clinical features of the HER2-low subgroup among HR+ early breast cancers.

Questions remain as to whether the HER2-low subgroup has its own distinct clinical and molecular features or whether it simply fits into the known spectrum of clinical subtypes or intrinsic subtypes. For clinical outcomes of HER2-low disease, the largest pooled analysis of patients undergoing neoadjuvant chemotherapy demonstrated lower pathologic complete response rates in hormone receptor–positive, HER2-low (HR+HER2-low) tumors compared with HR+HER2-zero tumors (17.5% *v* 23.6%);[6] however, this was not confirmed in a separate pooled analysis.[4] For survival outcomes, no significant difference has been demonstrated between HR+HER2-low and HR+HER2-zero tumors in several large pooled analyses and cohort studies.[4-9]

Regarding molecular features, a small number of studies have investigated gene expression differences between HR+HER2-low and HR+HER2-zero tumors. In an analysis of The Cancer Genome Atlas Breast, no significant difference in PAM50 classification was observed between HR+HER2-low and HR+HER2-zero subgroups with similar rates of luminal A (65.9% *v* 69.9%, respectively) and luminal B classifications (25.4% *v* 21.2%, respectively).[7] In addition, several studies have demonstrated significantly higher *ERBB2* transcript abundance in HR+HER2-low tumors compared with HR+HER2-zero tumors; however, the absolute difference appears to be small.[5,7] There is also higher expression of luminal-related genes in HR+HER2-low tumors compared with HR+HER2-zero tumors,[5] consistent with a separate finding of significant positive association of increasing estrogen receptor (ER) expression by IHC with increasing HER2-low frequency.[4] For differences in genomic alterations between HR+HER2-low and HR+HER2-zero tumors, in a subset of a pooled analysis comparing 268 HR+HER2-low with 129 HR+HER2-zero tumors, a lower frequency of *TP53* mutations was observed in HER2-low tumors (25% *v* 38%, respectively); however, no difference was observed for *PIK3CA* mutations (29% *v* 24%, respectively).[6] In a more recently published paper of 1,039 tumors from patients with HER2-negative metastatic breast cancer (mBC), 67.9% of whom had ER-positive cancers, there was no significant difference in the frequency of genomic alterations with the exception of a higher rate of *ERBB2* hemideletions in HER2-zero tumors.[10]

In this study, we sought to investigate clinical and genomic differences between HR+HER2-low and HR+HER2-zero early breast cancers to determine whether these are truly distinct biologic entities, using genomic sequencing data from two large randomized phase III clinical trials, BIG 1-98 and SOFT, both with central pathologic review of HER2 IHC status and patients who have not been exposed to previous endocrine therapies.

METHODS

Patients

This study includes patients with HR+HER2- early breast cancers who were enrolled in the phase III, randomized postmenopausal BIG 1-98 (ClinicalTrials.gov identifier: NCT00004205) and premenopausal SOFT (ClinicalTrials.gov identifier: NCT00066690) clinical trials. In these studies, patients were randomly assigned to adjuvant endocrine therapy (ET) treatment arms. Details of these trials have been previously reported.[11-14] This study includes patients from these clinical trials whose collected tumor samples had undergone genomic sequencing and had evaluable HER2 status by IHC as reviewed centrally (HER2-low *v* HER2-zero). Details of these translational research data sets,

including patient selection, have been previously reported for BIG 1-98[15,16] and SOFT.[17] All patients provided written informed consent. Ethics committees and relevant health authorities approved the protocol.

Next-Generation Sequencing

The included tumor samples from BIG 1-98 (n = 538 of 8,010 randomly assigned) underwent testing using the Foundation Medicine T5a panel, and tumor samples from SOFT (n = 1,276 of 3,066 randomly assigned) underwent testing using targeted DNA-sequencing using a customized hybridization capture panel for recurrent breast cancer genes (n = 1,257) and/or whole-exome sequencing (WES; n = 82). A list of target genes for the customized panel is available in the Data Supplement (Table S1).[18] Details on variant and copy number analysis have been previously published and include detection of short variants (point mutations, insertions, deletions), copy number alterations (amplifications, deletions), and selected fusions.[15,17]

Gene Expression Analysis

Gene expression data were available for a subset (n = 190 patients) from the BIG 1-98 trial. Formalin-fixed paraffin-embedded tumor samples underwent gene expression profiling using the Illumina Whole-Genome DASL HT bead array assay. Data were log base 2–transformed and normalized using the Illumina GenomeStudio Software Package. Only gene expression values for *ERBB2* were used in this analysis.

HER2-Low Status

Tumors were classified as HR+HER2-low if they had a centrally reviewed HER2 IHC score of 1+ or 2+ with negative ISH or HR+HER2-zero if they had an HER2 IHC score of 0. Central assessment of HER2 IHC was used for both the BIG 1-98 and SOFT cohorts using the polyclonal HercepTest.[19,20] Samples were excluded if HER2 was amplified by ISH or IHC status was unavailable; six tumor samples from SOFT were categorized on the basis of only local IHC assessment. In total, 520 and 1,275 samples were evaluable for this analysis from BIG 1-98 and SOFT, respectively (Fig 1). ER IHC, progesterone receptor (PgR) IHC, Ki-67 IHC, and grade were also centrally reviewed. In BIG 1-98, fluorescence in situ hybridization (FISH) was performed on a subset of tumors scored as HER2 IHC 2+ or 3+ or IHC 1+ with ≥50% immunoreactive cells or that were not assessable with IHC for technical reasons (eg, staining failure or detachment of the tissue section from slides during the assay).

Statistical Analysis

Comparisons between HER2-low and HER2-zero groups were performed using chi-squared tests (categorical variables) and t-tests and Mann-Whitney Wilcoxon tests (for normally and non-normally distributed continuous variables, respectively). For time-to-event analyses, the primary end point used was distant recurrence-free interval (DRFI). This is defined as the time from random assignment to recurrence at a distant site. Patients without a distant recurrence were censored at the date of last follow-up or death. Multivariable Cox proportional hazards regression models were used to assess associations of HER2-low status with DRFI and treatment-by-HER2-low status interaction. For BIG 1-98, the Cox model was stratified by treatment assignment and adjusted by tumor size, nodal status, and grade. For SOFT, the Cox model was stratified by nodal status and (neo)adjuvant chemotherapy receipt and adjusted by treatment assignment. The Kaplan-Meier method estimated the distribution of DRFI and 8-year freedom from distant recurrence. For assessment of genomic driver alteration frequency by HER2-low status, comparisons were performed using chi-squared tests and *P* values were adjusted for multiple testing using the false discovery rate method, with q values < 0.1 deemed significant. For all other tests, *P* values < .05 were deemed to be statistically significant. For BIG 1-98, patient samples were previously selected for genomic sequencing using a case-cohort–like selection approach. Full details on the statistical methods for the case-cohort–like selection approach in the BIG 1-98 translational study and the calculation of sampling weights have been previously published.[15,16] Therefore, all analyses including calculation of frequencies, comparison tests, and time-to-event analyses were performed using sampling weights using the generalized Horvitz-Thompson weighted method (inverse probability weighting).[21]

RESULTS

Patient Characteristics

Tumors that had undergone genomic testing and were evaluable for HER2-low status included 1,275 patient's tumors from the premenopausal SOFT clinical trial,[17] and 520 patient's tumor's from the post menopausal BIG 1-98 clinical trial (Fig 1A).[15] Nineteen tumors were excluded from the analysis (HER2-positive by FISH or IHC [n = 17], unavailable HER2 IHC status [n = 2]). Patient and tumor characteristics of the evaluable SOFT and BIG 1-98 patient cohorts are shown in Table 1.

Among the tumors from the postmenopausal BIG 1-98 HR+HER2- cohort, 203 (37% [24.4% IHC 1+, 12.2% IHC 2+]) were classified as HER2-low and 317 (63%) were classified as HER2-zero (Figs 1B and 1C). Among the tumors from the premenopausal SOFT cohort, 274 (21% [18.0% IHC 1+, 3.5% IHC 2+]) were classified as HER2-low and 1,001 (79%) were classified as HER2-zero (Figs 1B and 1C).

Clinicopathologic Associations With HER2-Low Status

The association between HER2-low status and clinicopathologic variables is summarized in Figure 2 and the Data Supplement (Table S2). There were no significant differences

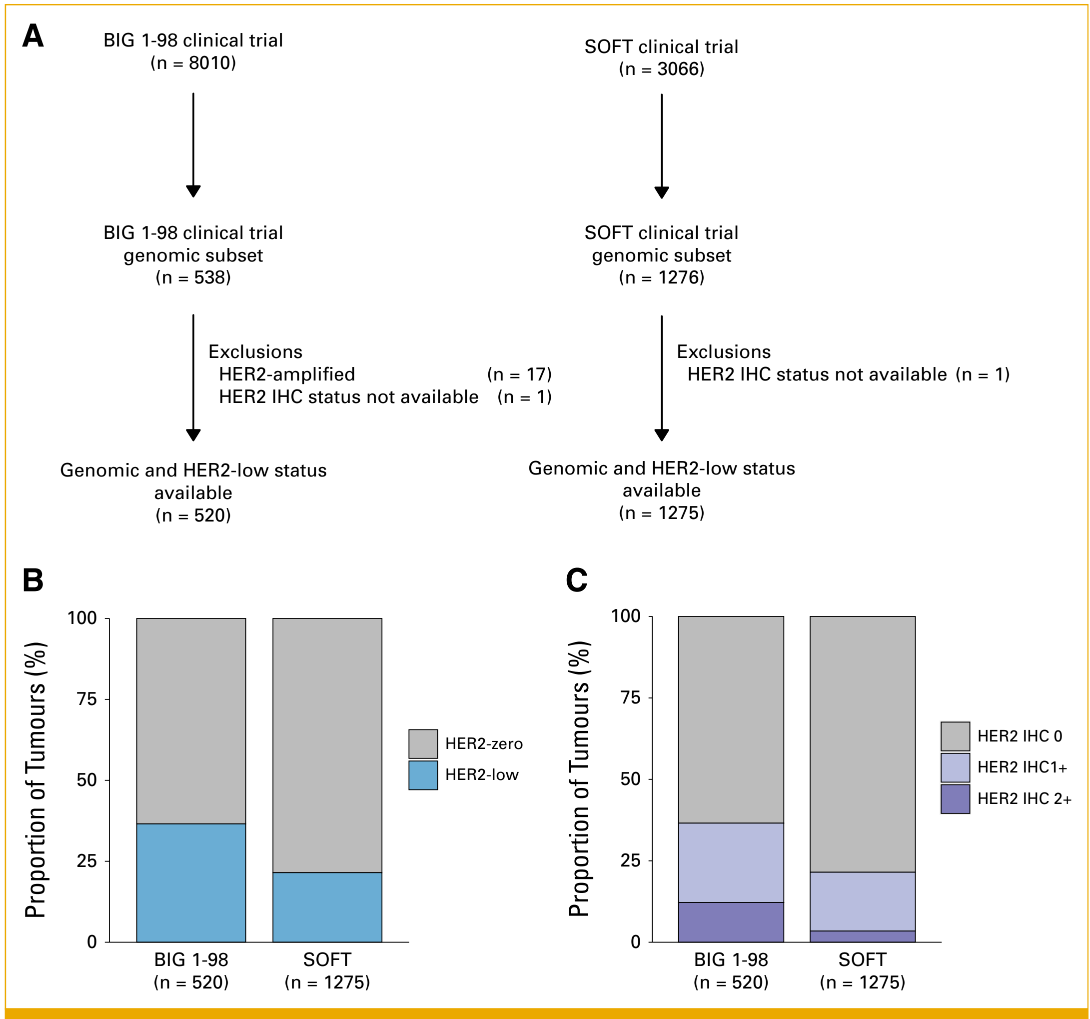

FIG 1. (A) Diagram demonstrating the number of tumors that were evaluable for this analysis from the postmenopausal BIG 1-98 and premenopausal SOFT clinical trial genomic subsets. (B) Bar chart displaying the proportion of evaluable tumors that were determined to be HER2-low in BIG1-98 and SOFT. (C) Bar chart displaying the proportion of evaluable tumors with corresponding HER2 IHC score in BIG 1-98 and SOFT. HER2 IHC assessment was performed in the central laboratory. IHC, immunohistochemistry; HER2, human epidermal growth factor receptor 2.

in tumor size or lymph node status by HER2-low status in either cohort. ER expression was significantly lower in HER2-zero tumors compared with HER2-low tumors from SOFT (median, 95% [IQR, 90-99] *v* 99% [IQR, 90-99], respectively, $P < .001$), but not in tumors from BIG 1-98 (median, 90% [IQR, 85-95] *v* 95% [IQR, 90-99], respectively, $P = .23$; Fig 2A). There was no significant difference in PgR expression by HER2-low status in either cohort. The distribution of tumor grade was significantly lower in HER2-zero tumors compared with HER2-low tumors from BIG 1-98 (17% *v* 29% grade 3, respectively, $P = .003$), but the grade distributions did not differ in tumors from SOFT (19% *v* 22% grade 3, $P = .13$; Fig 2C). Similarly, Ki-67 levels were significantly lower in HER2-zero tumors compared with HER2-low tumors from BIG 1-98 (median, 14% [IQR, 6-20] *v* 16% [IQR, 10-25], respectively, $P < .001$), but not in the SOFT cohort (median, 17 [IQR, 11-23] *v* 17.5% [IQR, 12-24], $P = .11$; Fig 2D). There was no significant association between the proportion of HER2-low tumors and increasing age categories within either pre- or postmenopausal cohorts (Fig 3A).

We next evaluated associations between HER2-low status and risk of distant recurrence. There was no significant difference in the risk of distant recurrence for HER2-low tumors compared with HER2-zero tumors in BIG-198 (multivariable hazard ratio [HR], 0.89 [95% CI, 0.58 to 1.38]; $P = .61$; 5-year distant recurrence-free (%) 94.0% *v* 92.8%, respectively) or in SOFT (multivariable HR, 1.23 [95% CI, 0.20 to 1.84]; $P = .31$; 5-year distant recurrence-free (%) 89.4% *v* 92.7%, respectively; Figs 3B and 3C). An exploratory analysis of BIG 1-98 demonstrated no significant difference in efficacy of letrozole compared with tamoxifen between HER2-low and HER2-zero tumors in BIG 1-98 ($P_{int} = .24$) and no significant difference in efficacy for the addition of ovarian function suppression to tamoxifen compared with tamoxifen alone between HER2-low and HER2-zero tumors in SOFT ($P_{int} = .36$; Data Supplement, Fig S1). There was no

TABLE 1. Patient and Tumor Characteristics for the Population That is Evaluable for This Analysis

Clinicopathological Variable	BIG 1-98 (n = 520)	SOFT (n = 1,275)
Age group, years, No. (%)		
<35		123 (10)
35-39		236 (19)
40-44	5 (<1)	383 (30)
45-49	28 (6)	390 (31)
50-54	75 (15)	140 (110)
55-59	109 (26)	3 (<1)
60-64	113 (20)	
≥65	190 (32)	
Menopausal status at trial random assignment, No. (%)		
Postmenopausal	520 (100)	0 (0)
Premenopausal	0 (0)	1,275 (100)
Tumor size, No. (%)		
≤2 cm	299 (64)	833 (65)
>2 cm	234 (36)	421 (33)
Unknown	5 (<1)	21 (17)
Nodal status, No. (%)		
Positive	289 (36)	431 (34)
Negative	249 (64)	84 (66)
Unknown	0	3 (<1)
Grade, No. (%)		
1	74 (21)	312 (25)
2	293 (57)	715 (56)
3	17 (22)	248 (19)
Unknown	2 (<1)	1 (<1)
ER expression, median (IQR)	95 (90-99)	95 (90-99)
PgR expression, median (IQR)	70 (10-90)	90 (70-99)
Ki-67 expression, median (IQR)	15 (8-23)	17 (11-23)

NOTE. Numbers in brackets are percentages of each population with the exception of age where the numbers in brackets represent the age range. For BIG 1-98, the proportions displayed are weighted proportions. ER expression, PgR expression, and grade are all centrally assessed values.
Abbreviations: ER, estrogen receptor; PgR, progesterone receptor.

significant outcome differences according to young versus very young (>40 years *v* ≤40 years) in the premenopausal SOFT cohort.

Genomic Associations With HER2-Low Status

We next assessed for differences in frequencies of genomic driver alterations by HER2-low status using genes that were altered at a frequency of at least 5% in either cohort. Overall frequencies of genomic driver alterations were similar between HER2-low and HER2-zero tumors in both cohorts (Fig 4, Data Supplement, Table S3). Mutations in *MAP3K1* were the only alteration that was at a lower frequency in HER2-low tumors compared with HER2-zero tumors in both BIG 1-98 (5% *v* 19%, q = 0.002) and SOFT (6% *v* 11%, q = 0.080). Mutations in *BRCA2* were significantly more frequent in HER2-low tumors compared with HER2-zero tumors in SOFT (9% *v* 5%, q = 0.078), but not in BIG 1-98 (4% *v* 1%, q = 0.22). Amplifications in *FGFR1* were significantly more frequent in HER2-low tumors compared with HER2-zero tumors in SOFT (15% *v* 9%, q = 0.073), but was not in BIG 1-98 (11% *v* 7%, q = 0.55). Whole-genome copy number profiles did not appear to be different between HER2-low and HER2-zero tumors (Data Supplement, Fig S2).

Genomic copy number of *ERBB2* between HER2-low and HER2-zero tumors using copy number segmentation data was then assessed. HER2-low tumors had significantly higher ERBB2 copy number segmentation values than HER2-zero tumors in both BIG 1-98 (median LogR 0.30 *v* 0.22, *P* < .001) and SOFT (median LogR 0.03 *v* 0.00, *P* < .001); however, absolute differences were small with a significant overlap of values (Fig 5A). Similarly, significantly higher ERBB2 copy number segmentation values were observed with increasing ERBB2 IHC values in both BIG 1-98 (*P* < .001) and SOFT (*P* < .001), again with only small absolute differences between groups (Data Supplement, Fig S3). Given the

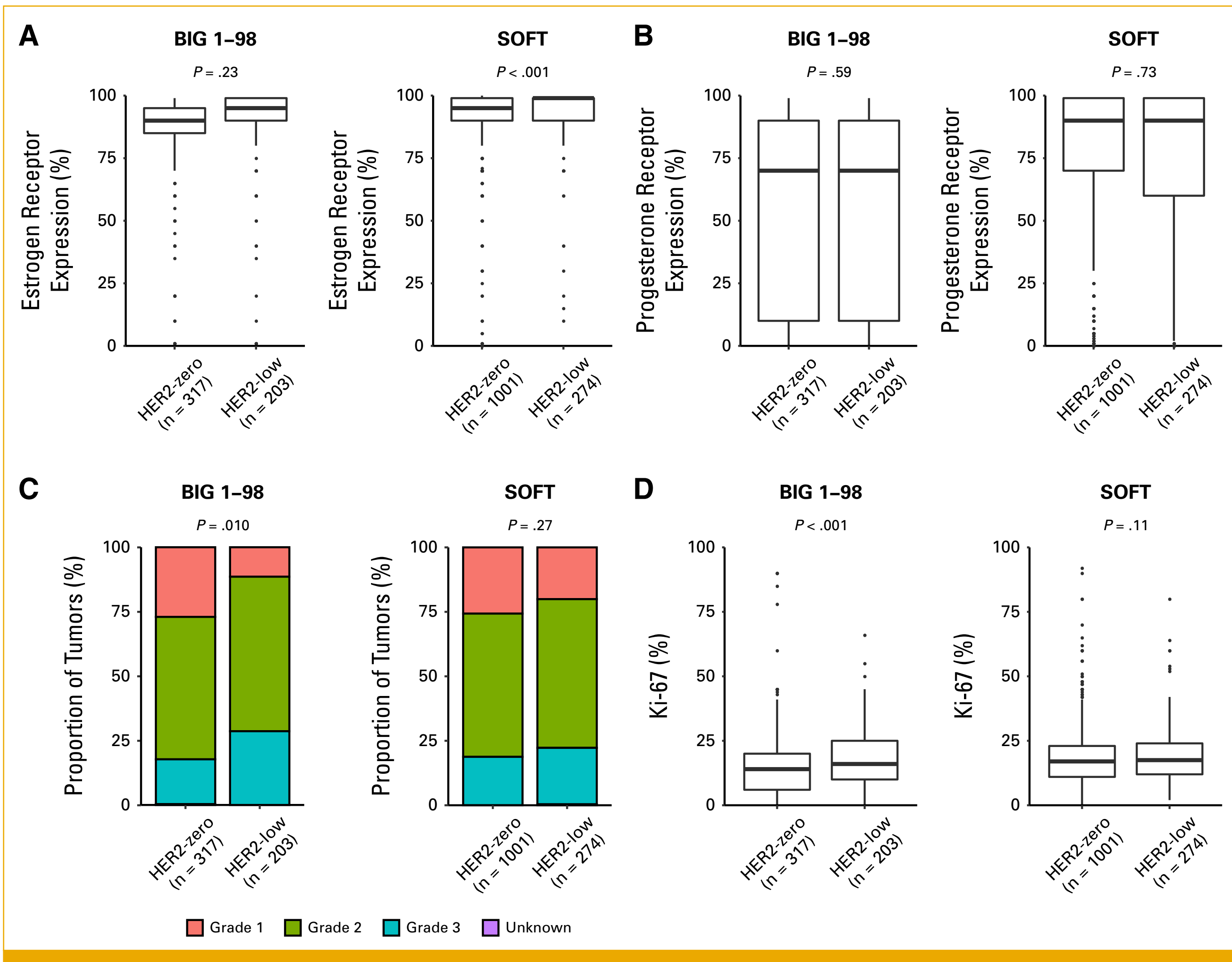

FIG 2. (A) Box plots demonstrating centrally assessed % tumor ER IHC expression by HER2-low status. (B) Box plots demonstrating centrally assessed % tumor PgR IHC expression by HER2-low status. (C) Bar plot demonstrating the proportion of tumors classified as centrally assessed grade one, two, three, or unknown by HER2-low status. (D) Box plots demonstrating centrally assessed % Ki-67 IHC expression by HER2-low status. ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; PgR, progesterone receptor.

small absolute differences in values, we considered it unlikely that HER2-low tumors had a globally increased tumor ERBB2 copy number compared with HER2-zero tumors; however, heterogeneous tumor cell increase in ERBB2 copy number may be possible. Concordant with this, a subsample of 73 tumors from SOFT, which had undergone WES and had allele-specific copy number data available, demonstrated no significant difference in integer copy number of ERBB2 between HER2-low and HER2-zero tumors (P = .85) and between ERBB2 IHC groups (P = .84; Data Supplement, Fig S4). In the BIG 1-98 cohort with available ERBB2 IHC percentage tumor staining data (n = 203), there was a high level of heterogeneous staining in the HER2-low group (Data Supplement, Fig S5).

ERBB2 gene expression was then assessed between HER2-low and HER2-zero tumors using a subset of tumors with gene expression data available from BIG 1-98 (n = 190; Fig 5B). HER2-low tumors had significantly higher *ERBB2* gene expression values than HER2-zero tumors in this subset (median 0.07 *v* −0.18, $P < .001$); however, again, absolute differences were small with a significant overlap of values between the subgroups. Similarly, significantly increasing *ERBB2* gene expression values were observed with increasing ERBB2 IHC values in this subset (P = .002; Data Supplement, Fig S6). Given the high levels of overlap in *ERBB2* gene expression values between these groups, it did not appear that the subgroups defined by ERBB2 IHC captured distinct ERBB2 expressing groups, at least at the transcriptional level. Correlation between *ERBB2* copy number values and gene expression values was modest at best (r = 0.17; Fig 5C).

DISCUSSION

In this analysis of 1,795 HR+HER2- early breast cancers from the BIG 1-98 and SOFT randomized phase III clinical trials of adjuvant ET in postmenopausal and premenopausal

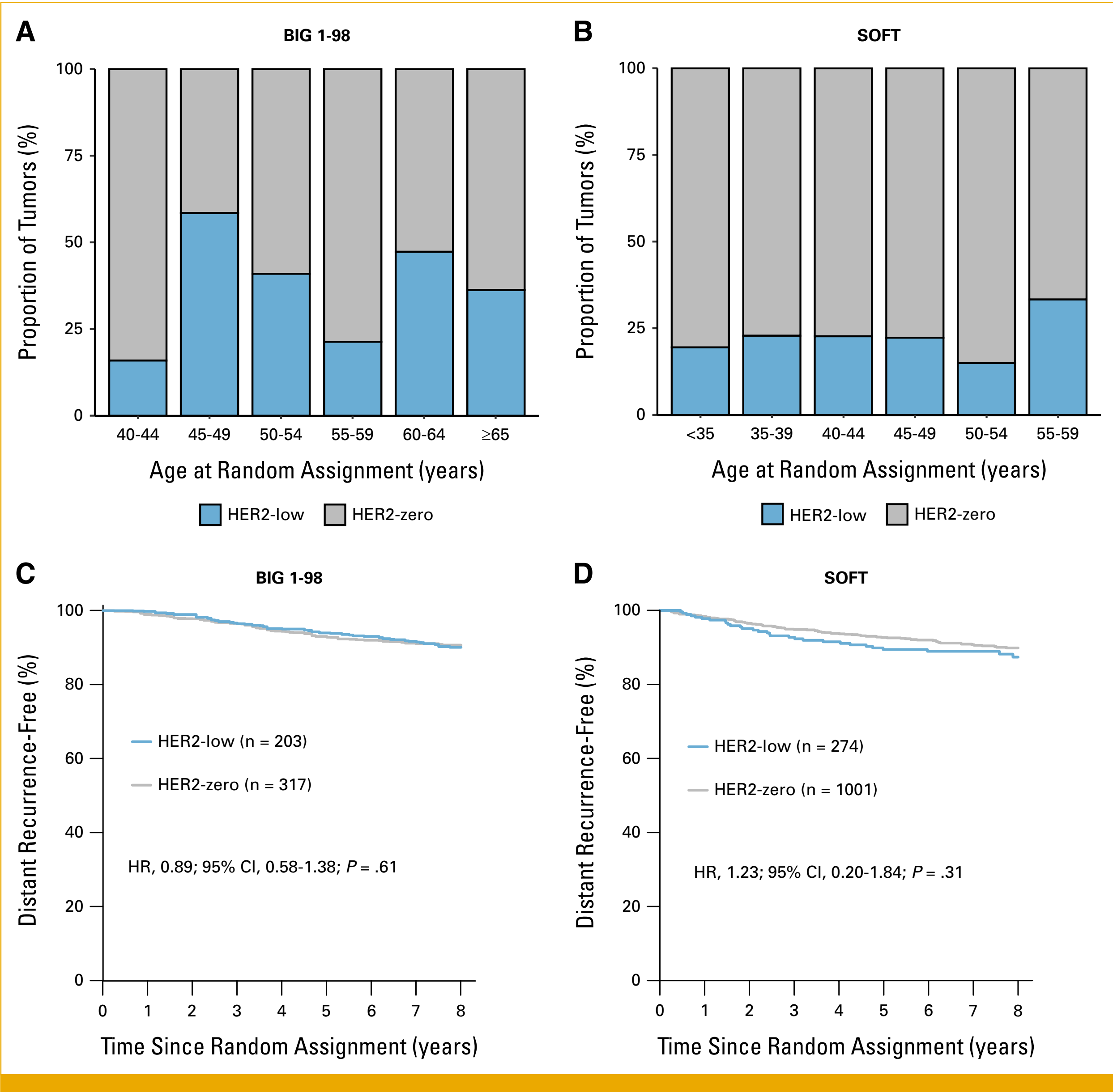

FIG 3. (A) Bar plot demonstrating the proportion of HER2-low tumors and HER2-zero tumors by age groups in BIG 1-98. (B) Bar plot demonstrating the proportion of HER2-low tumors and HER2-zero tumors by age group in the premenopausal SOFT cohort. (C) Kaplan-Meier estimate of DRFI by HER2-low status in the postmenopausal BIG 1-98 cohort. (D) Kaplan-Meier estimate of DRFI by HER2-low status in the SOFT premenopausal cohort. The HR characterizes HER2-low relative to HER2-zero. HR, hazard ratio; DRFI, distant recurrence-free interval; HER2, human epidermal growth factor receptor 2.

patients, respectively, we investigated differences in genomic profiles between HR+HER2-low and HR+HER2-zero tumors. We found similar genomic profiles between HR+HER2-low and HR+HER2-zero tumors, suggesting that these are not distinct entities at the genomic level. The only exception to this was mutations in *MAP3K1*, which were found at higher frequencies in HR+HER2-zero tumors, but were not exclusive to HR+HER2-zero tumors. These results are similar to a recently published cohort that included patients with HER2-negative mBC.[10] While we observed that *ERBB2* copy number value was significantly higher in HR+HER2-low tumors compared with HR+HER2-zero tumors, the absolute difference between these entities was small with large overlaps in the range of values between the two groups, suggesting that these categories do not make up distinct subgroups at the *ERBB2* copy number level. In a subset of tumors in postmenopausal BIG 1-98 with *ERBB2* gene expression data, we found similar findings which have been demonstrated in other published cohorts.[5,7] In this small subset, the correlation between *ERBB2* copy number and gene expression was poor among these tumors, suggesting that expression of *ERBB2* may be influenced by other factors beyond gene copy number. Alternatively, it is possible that these small differences may be due to

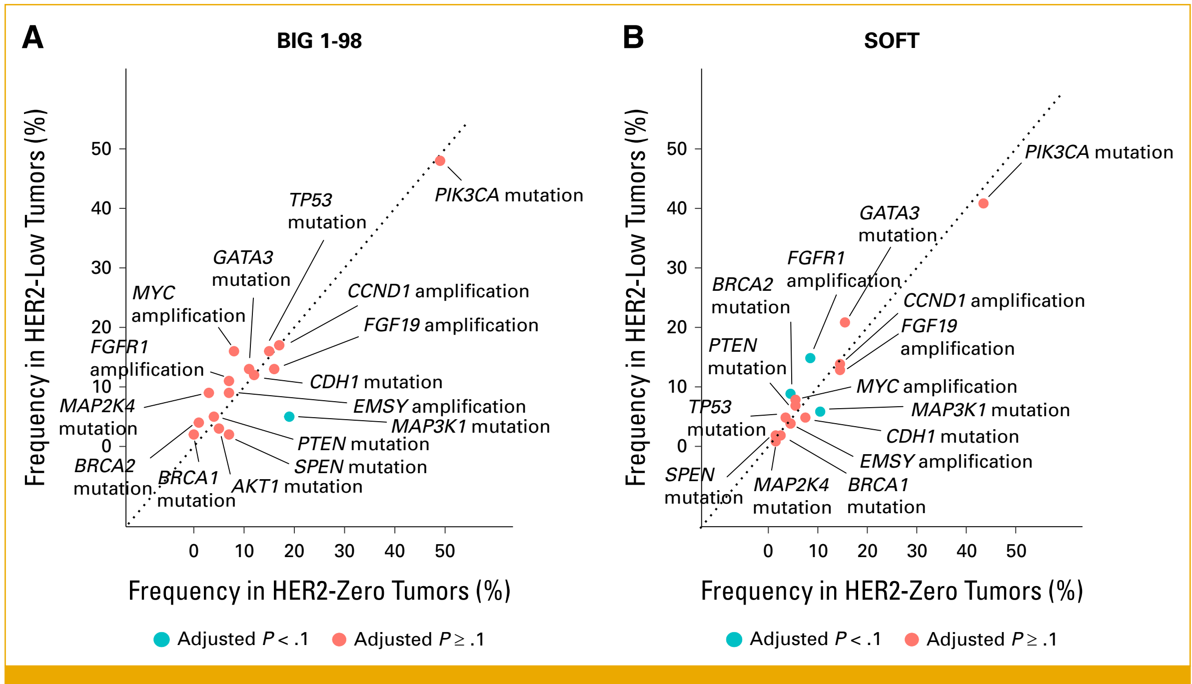

FIG 4. (A) Plot demonstrating the frequency of genomic alteration in HER2-low versus HER2-zero tumors in the postmenopausal BIG 1-98 trial cohort. (B) Plot demonstrating the frequency of genomic alteration in HER2-low versus HER2-zero tumors in the premenopausal SOFT trial cohort. HER2, human epidermal growth factor receptor 2.

heterogeneous *ERBB2* copy number gains in tumor subpopulations which cannot be clearly defined in bulk sequencing data.

From a clinical perspective, we observed higher median ER expression in HER2-low tumors compared with HER2-zero tumors in SOFT; however again, the absolute difference was small (median 99% *v* 95%). This is consistent with other studies which have demonstrated higher expression in luminal genes in HER2-low tumors and a higher frequency of HER2-low tumors with increasing ER IHC expression.[4,5] There were no other differences in clinicopathologic variables between the HR+HER2-low and HR+HER2-zero subgroups, including the risk of distant recurrence after an 8-year median follow-up. Interestingly, the frequency of HR+ HER2-low tumors was higher in the postmenopausal BIG 1-98 cohort than our premenopausal SOFT cohort; however, the frequencies may be influenced by differences in each trial's eligibility criteria, and confirmation in other data sets is required.

Questions still remain as to what factors mediate ERBB2 expression in HR+ HER2-low breast cancer. Unlike in HER2-amplified breast cancer where ERBB2 expression is via genomic copy number amplification of *ERBB2*, a strong oncogenic driver of tumor growth, our data did not identify any genomic driver that is clearly associated with increased ERBB2 expression in HER2-low disease. It is therefore unsurprising that tumor ERBB2 expression without HER2 amplification is subject to dynamic change over time and influenced by treatment selection, a finding that was reported in two separate studies which identified high levels of discordance in HER2-low status between primary and metastatic samples, particularly in HR+ tumors and with a higher rate of conversion to HER2-low disease in the metastatic setting, with the caveat that these samples might not have been centrally reviewed.[22,23] On the basis of this, further investigation into the factors mediating dynamic ERBB2 expression are warranted. Given the known poor interpathologist reproducibility in HER2 IHC assessment in non–HER2-amplified tumors,[1] it is prudent to exercise caution in drawing robust conclusions on research findings without central pathology review. Further research into more quantitative measures of HER2 protein expression is warranted and is especially relevant to treatment and resistance to trastuzumab deruxtecan and other ADCs targeting HER2 in breast cancer and other solid tumor types.

Finally, our data support the importance of further research toward improving patient selection biomarkers for HER2-directed ADCs, noting that each HER2-directed ADC has unique properties that may affect its efficacy, particularly in HER2-low disease. For example, trastuzumab deruxtecan has a high drug-to-antibody ratio of 8 and additional features of a hydrolysable linker plus a membrane permeable function of the topoisomerase inhibitor payload, rendering it capable of on- and off-target tumor microenvironment effects. This contrasts with T-DM1 with a lower drug-to-antibody ratio of 2 and without additional features. A major benefit for an IHC-based score is the ease with which it can be applied in any pathology setting since it is already standardized. However, HER2 IHC and scoring were initially

© 2025 by American Society of Clinical Oncology

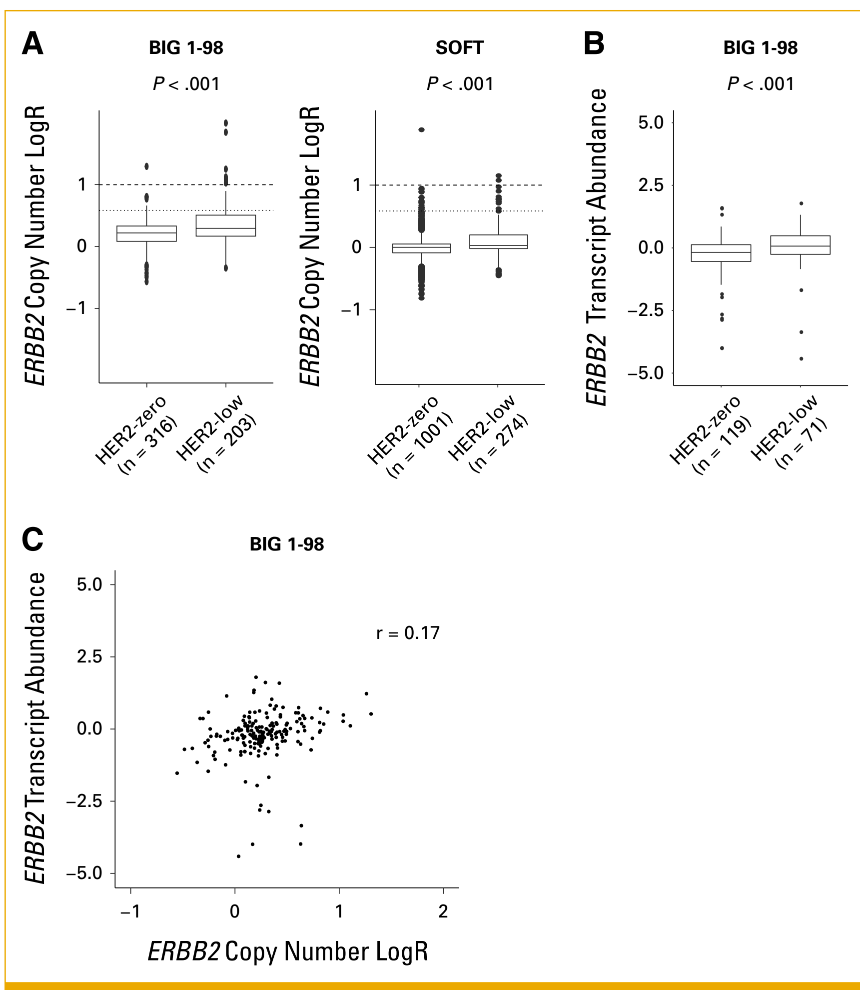

FIG 5. (A) Boxplot demonstrating the *ERBB2* copy number LogR by HER2-low status according to the trial cohort. The dotted horizontal line represents estimated LogR value for low-level ERBB2 copy number gain (approximately 4 copies), and the dashed horizontal line represents the estimated LogR value for ERBB2 amplification (approximately 6 copies). (B) Boxplot demonstrating the *ERBB2* transcript abundance by HER2-low status in postmenopausal BIG 1-98. (C) Plot demonstrating the relationship between *ERBB2* transcript abundance and *ERBB2* copy number LogR in BIG 1-98. HER2, human epidermal growth factor receptor 2.

designed to identify HER2-amplified disease with high sensitivity and specificity[1] and have limited reproducibility among pathologists at lower levels of IHC staining.[24] Recently updated ASCO-College of American Pathologists recommendations for HER2 testing acknowledged the need for ongoing HER2 IHC reporting, but without defining a category as "HER2-low" at this stage given the limited data for activity of trastuzumab deruxtecan in patients with HER2 IHC 0 tumors.[25] In addition, they provide practical suggestions to aid pathologists in accurately distinguishing HER2 IHC categories. Other new technologies may provide improved granularity for assessment of HER2 protein quantification in the future (eg, mass spectroscopy). Our data and data of others suggest that there is considerable overlap in copy number status and *ERBB2* gene expression between HR+HER2-low and HR+HER2-zero tumors, implying that use of the HER2-low definition by IHC may not reliably select patients who could benefit from trastuzumab deruxtecan. Consistent with this notion, the phase II DAISY clinical trial recruited a cohort of heavily pretreated patients with HER2-zero tumors and demonstrated objective response rates of 29.7%.[26] In addition, the DESTINY-Breast06 study which included 152 patients with HER2-ultralow breast cancers (HER2 IHC 0 but with faint, incomplete membrane staining in ≤10% of tumor cells) demonstrated progression-free survival (PFS) improvement with trastuzumab deruxtecan compared with treatment of physician's choice, consistent with the PFS improvement observed in HER2-low tumors.[27] Given the improved overall survival benefit for trastuzumab deruxtecan versus physician's choice of chemotherapy in the DESTINY-Breast04 and DESTINY-Breast06 studies,[2,27] it should be considered whether patients with HER2-zero

tumors may be missing out on an effective treatment because of an inaccurate predictive biomarker.

In conclusion, this analysis of 1,795 HR+ early breast cancers from postmenopausal BIG 1-98 and premenopausal SOFT phase III clinical trials does not support HR+HER2-low breast cancer as a distinct clinical or biologic entity. Strengths of this study include robust quality clinical data and long-term survival outcomes from two phase III, randomized clinical trials and centrally determined HER2 IHC status. Limitations include retrospective design and the fact that tumors were evaluated in the early breast cancer rather than advanced setting. Tumor mutation burden testing was not standard or validated for the used assays and is therefore not reported. In addition, different next generation sequencing assays were used for each of the clinical trials presented here, making comparisons between the studies difficult. At the time of central IHC assessment, the concept of HER2-low breast cancer was not known and this might have affected reporting. Because of this, the HER2-ultra low classification was not included in this analysis. Nevertheless, our data suggest that ongoing efforts into optimization of patient selection for HER2-directed ADC therapies are warranted. This may be crucially important as the development of these drugs moves into the early-stage, operable disease setting.

AFFILIATIONS

[1]Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia
[2]Sir Peter MacCallum Department of Oncology, The University of Melbourne, Victoria, Australia
[3]International Breast Cancer Study Group, Coordinating Center, Central Pathology Office, Bern, Switzerland
[4]International Breast Cancer Study Group Central Pathology Office, Milan, Italy
[5]Department of Pathology and Laboratory Medicine, IEO European Institute of Oncology IRCCS, Milan, Italy
[6]International Breast Cancer Study Group and University of Sydney, Sydney, Australia
[7]Department of Data Science, Dana-Farber Cancer Institute, Harvard Medical School, Harvard T.H. Chan School of Public Health, and Frontier Science and Technology Research Foundation, Boston, MA
[8]SwissBreastCare, Zürich, Switzerland
[9]Swiss Group for Clinical Cancer Research SAKK, Berne, Switzerland
[10]Division of Medical Senology, IEO, European Institute of Oncology IRCCS, Milan, Italy
[11]Department of Medicine, The University of Chicago, Chicago, IL
[12]Division of Biostatistics, International Breast Cancer Study Group Statistical Center, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

CORRESPONDING AUTHOR

Sherene Loi, MD, PhD; e-mail: sherene.loi@petermac.org.

SUPPORT

For this translational research project, the SOFT trial cohort DNA sequencing was supported by a Susan G. Komen for the Cure Promise Grant (grant Q15 number CCR14299387 to S.J.L.), the National Health and Research Council (NHMRC) Australia (grant 1084531), Breast Cancer Research Foundation, NBCF Australia, and the family of Judy Eisman in Australia. The BIG 1-98 trial cohort DNA sequencing at Foundation Medicine was supported with funding by Novartis; assessment of gene expression was supported with funding by Susan G. Komen for the Cure Promise Grant (grant KG080081). Tissue collection and central pathology of the trials was supported by Susan G. Komen for the Cure Promise Grant (grant nubmer KG080081), the Breast Cancer Research Foundation, Novartis, and the Alliance for Clinical Trials in Oncology (for SOFT; US NIH U10CA180821). The SOFT clinical trial received financial support for trial conduct from Pfizer, the International Breast Cancer Study Group (IBCSG), the US National Cancer Institute, including the lead group Alliance for Clinical Trials in Oncology (Alliance; US NIH U10CA180821), the Breast Cancer Research Foundation (CONS 23-011), Ipsen, TerSera, and AstraZeneca. Pfizer and Ipsen provided drug supply. The BIG 1-98 clinical trial received financial support for trial conduct from Novartis, including drug supply. Additional support to the coordinating group, IBCSG, for conduct of the clinical trials included: Frontier Science and Technology Research Foundation, Swiss Group for Clinical Cancer Research (SAKK), Cancer Research Switzerland, Oncosuisse, Cancer League Switzerland, Foundation for Clinical Cancer Research of Eastern Switzerland (OSKK), US National Cancer Institute (US NIH grant CA075362), The Cancer Council Australia, Breast Cancer Trials Australia and New Zealand; and additional support to Breast Cancer Trials Australia & New Zealand for SOFT (NHMRC grant 351161, 510788, 1105058 and Breast Cancer Research Foundation CONS-23-001 for Bio-Bank).

DATA SHARING STATEMENT

A data sharing statement provided by the authors is available with this article at DOI https://doi.org/10.1200/PO-24-00599. After publication, access to deidentified participant data may be requested by researchers by submitting a proposal to stat_center@ibcsg.org, which will be reviewed for scientific merit and feasibility in accordance with the Guidelines for Collaborative research and data sharing policy for IBCSG trials (https://www.ibcsg.org/en/patients-professionals/research-collaboration).

AUTHOR CONTRIBUTIONS

Conception and design: Stephen J. Luen, Alan S. Coates, Meredith M. Regan, Sherene Loi
Administrative support: Roswitha Kammler
Provision of study materials or patients: Olivia Biasi, Beat Thürlimann, Prudence A. Francis
Collection and assembly of data: Stephen J. Luen, Peter Savas, Roswitha Kammler, Patrizia Dell'Orto, Olivia Biasi, Beat Thürlimann, Marco Colleoni, Prudence A. Francis, Meredith M. Regan, Giuseppe Viale, Sherene Loi
Data analysis and interpretation: Stephen J. Luen, Lauren C. Brown, Courtney T. van Geelen, Richard D. Gelber, Beat Thürlimann, Gini F. Fleming, Prudence A. Francis, Meredith M. Regan, Sherene Loi
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

© 2025 by American Society of Clinical Oncology

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/PO/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Stephen J. Luen
Honoraria: AstraZeneca
Research Funding: Roche (Inst), BeiGene (Inst), Novartis (Inst), SpringWorks Therapeutics (Inst), AstraZeneca (Inst)
Travel, Accommodations, Expenses: AstraZeneca

Lauren C. Brown
Travel, Accommodations, Expenses: AstraZeneca

Peter Savas
Stock and Other Ownership Interests: Elevance Health

Patrizia Dell'Orto
Honoraria: MSD Oncology (I), Pfizer (I)
Consulting or Advisory Role: Roche/Genentech (I)
Speakers' Bureau: Roche/Genentech (I)
Research Funding: Roche/Genentech (I)
Travel, Accommodations, Expenses: Roche (I)

Alan S. Coates
Stock and Other Ownership Interests: Avita Medical Inc, CSL Limited, Ramsay Health Care, Resmed

Richard D. Gelber
Research Funding: AstraZeneca (Inst), Roche (Inst), Merck (Inst)

Beat Thürlimann
Employment: Innomedica
Stock and Other Ownership Interests: Roche (I), Novartis (I), Alcon, Merck, Sandoz, InnoMedica (I)
Consulting or Advisory Role: enzymmanagment, Gilead Sciences, PageTherapeutics, Breastceuticals, Menarini
Expert Testimony: AstraZeneca/Daiichi Sankyo

Gini F. Fleming
Research Funding: Corcept Therapeutics (Inst), Abbvie (Inst), Iovance Biotherapeutics (Inst), Syros Pharmaceuticals (Inst), Sermonix Pharmaceuticals (Inst), Compugen (Inst), Plexxikon (Inst), Roche (Inst), GlaxoSmithKline (Inst), Celldex (Inst), AstraZeneca (Inst), Molecular Templates (Inst), CytomX Therapeutics (Inst), Astellas Pharma (Inst), K-Group Beta (Inst), AstraZeneca (Inst), Compugen (Inst), Merck (Inst), Incyte (Inst), Pfizer (Inst), Artios (Inst), Blueprint Medicines (Inst), Duality Biologics (Inst), Sanofi (Inst)
Other Relationship: DSI (Inst), Merck (Inst), Caris Life Sciences (Inst), Eisai (Inst), AstraZeneca (Inst)

Prudence A. Francis
Honoraria: Lilly, Amplity Health

Meredith M. Regan
Honoraria: Bristol Myers Squibb, Merck
Consulting or Advisory Role: Tolmar, Bristol Myers Squibb, Tersera
Research Funding: Pfizer (Inst), Novartis (Inst), Bayer (Inst), Bristol Myers Squibb (Inst), Roche (Inst), TerSera (Inst), bioTheranostics (Inst)

Giuseppe Viale
Honoraria: Daiichi Sankyo Europe GmbH, Gilead/47
Consulting or Advisory Role: Dako, Roche/Genentech, Novartis, Daiichi Sankyo, MSD Oncology, Menarini, AstraZeneca (I)
Research Funding: Roche/Genentech (Inst), Ventana Medical Systems (Inst), Dako/Agilent Technologies (Inst), Cepheid (Inst), AstraZeneca (Inst)
Travel, Accommodations, Expenses: AstraZeneca, Daiichi Sankyo Europe GmbH

Sherene Loi
Honoraria: Roche/Genentech, Novartis, MSD Oncology, Mersana (I)
Consulting or Advisory Role: Roche/Genentech (Inst), Aduro Biotech (Inst), Novartis (Inst), G1 Therapeutics (Inst), PUMA Biotechnology (Inst), GlaxoSmithKline (Inst), AstraZeneca (Inst), Seagen (Inst), BMS (Inst), Silverback Therapeutics (Inst), Pfizer (Inst), Gilead Sciences (Inst), Gilead Sciences (Inst), Daiichi Sankyo/Lilly (Inst), Tallac Therapeutics (Inst)
Research Funding: Roche/Genentech (Inst), Novartis (Inst), Merck (Inst), Puma Biotechnology (Inst), Bristol Myers Squibb (Inst), Seagen (Inst), AstraZeneca (Inst), Nektar (Inst), Lilly (Inst)
Travel, Accommodations, Expenses: BMS
Other Relationship: Roche Medical writing support

No other potential conflicts of interest were reported.

ACKNOWLEDGMENT

We are indebted to the women, physicians, nurses, trial coordinators, and data managers who participated in the SOFT and BIG 1-98 clinical trials and the many pathologists who submitted tumor and normal tissue blocks for research purposes; to the IBCSG Central Pathology Office for tumor block collection; and to the International Breast Cancer Study Group (IBCSG) for the design and conduct of the trials. The trials were sponsored by the IBCSG and ETOP IBCSG Partners Foundation and conducted in collaboration with the Breast International Group (BIG) and BIG cooperative groups. SOFT was also conducted in collaboration with the US NCI National Clinical Trials Network cooperative groups.

REFERENCES

1. Tarantino P, Hamilton E, Tolaney SM, et al: HER2-low breast cancer: Pathological and clinical landscape. J Clin Oncol 38:1951-1962, 2020
2. Modi S, Jacot W, Yamashita T, et al: Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. N Engl J Med 387:9-20, 2022
3. Fehrenbacher L, Cecchini RS, Geyer CE Jr, et al: NSABP B-47/NRG Oncology phase III randomized trial comparing adjuvant chemotherapy with or without trastuzumab in high-risk invasive breast cancer negative for HER2 by FISH and with IHC 1+ or 2. J Clin Oncol 38:444-453, 2020
4. Tarantino P, Jin Q, Tayob N, et al: Prognostic and biologic significance of ERBB2-low expression in early-stage breast cancer. JAMA Oncol 8:1177-1183, 2022
5. Schettini F, Chic N, Braso-Maristany F, et al: Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. NPJ Breast Cancer 7:1, 2021
6. Denkert C, Seither F, Schneeweiss A, et al: Clinical and molecular characteristics of HER2-low-positive breast cancer: Pooled analysis of individual patient data from four prospective, neoadjuvant clinical trials. Lancet Oncol 22:1151-1161, 2021
7. Agostinetto E, Rediti M, Fimereli D, et al: HER2-low breast cancer: Molecular characteristics and prognosis. Cancers (Basel) 13:2824, 2021
8. Gampenrieder SP, Rinnerthaler G, Tinchon C, et al: Landscape of HER2-low metastatic breast cancer (MBC): Results from the Austrian AGMT_MBC-Registry. Breast Cancer Res 23:112, 2021
9. Won HS, Ahn J, Kim Y, et al: Clinical significance of HER2-low expression in early breast cancer: A nationwide study from the Korean Breast Cancer Society. Breast Cancer Res 24:22, 2022
10. Tarantino P, Gupta H, Hughes ME, et al: Comprehensive genomic characterization of HER2-low and HER2-0 breast cancer. Nat Commun 14:7496, 2023
11. Francis PA, Regan MM, Fleming GF, et al: Adjuvant ovarian suppression in premenopausal breast cancer. N Engl J Med 372:436-446, 2015
12. Francis PA, Pagani O, Fleming GF, et al: Tailoring adjuvant endocrine therapy for premenopausal breast cancer. N Engl J Med 379:122-137, 2018
13. Breast International Group BIG 1-98 Collaborative Group; Thürlimann B, Keshaviah A, et al: A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. N Engl J Med 353:2747-2757, 2005

14. Regan MM, Neven P, Giobbie-Hurder A, et al: Assessment of letrozole and tamoxifen alone and in sequence for postmenopausal women with steroid hormone receptor-positive breast cancer: The BIG 1-98 randomised clinical trial at 8·1 years median follow-up. Lancet Oncol 12:1101-1108, 2011
15. Luen SJ, Asher R, Lee CK, et al: Association of somatic driver alterations with prognosis in postmenopausal, hormone receptor-positive, HER2-negative early breast cancer: A secondary analysis of the BIG 1-98 randomized clinical trial. JAMA Oncol 4:1335-1343, 2018
16. Luen SJ, Asher R, Lee CK, et al: Identifying oncogenic drivers associated with increased risk of late distant recurrence in postmenopausal, estrogen receptor-positive, HER2-negative early breast cancer: Results from the BIG 1-98 study. Ann Oncol 31:1359-1365, 2020
17. Luen SJ, Viale G, Nik-Zainal S, et al: Genomic characterisation of hormone receptor-positive breast cancer arising in very young women. Ann Oncol 34:397-409, 2023
18. Van Geelen CT, Savas P, Teo ZL, et al: Clinical implications of prospective genomic profiling of metastatic breast cancer patients. Breast Cancer Res 22:91, 2020
19. Rasmussen BB, Regan MM, Lykkesfeldt AE, et al: Adjuvant letrozole versus tamoxifen according to centrally-assessed ERBB2 status for postmenopausal women with endocrine-responsive early breast cancer: Supplementary results from the BIG 1-98 randomised trial. Lancet Oncol 9:23-28, 2008
20. Regan MM, Pagani O, Francis PA, et al: Predictive value and clinical utility of centrally assessed ER, PgR, and Ki-67 to select adjuvant endocrine therapy for premenopausal women with hormone receptor-positive, HER2-negative early breast cancer: TEXT and SOFT trials. Breast Cancer Res Treat 154:275-286, 2015
21. Gray RJ: Weighted analyses for cohort sampling designs. Lifetime Data Anal 15:24-40, 2009
22. Miglietta F, Griguolo G, Bottosso M, et al: Evolution of HER2-low expression from primary to recurrent breast cancer. NPJ Breast Cancer 7:137, 2021
23. Tarantino P, Gandini S, Nicolo E, et al: Evolution of low HER2 expression between early and advanced-stage breast cancer. Eur J Cancer 163:35-43, 2022
24. Baez-Navarro X, Salgado R, Denkert C, et al: Selecting patients with HER2-low breast cancer: Getting out of the tangle. Eur J Cancer 175:187-192, 2022
25. Wolff AC, Somerfield MR, Dowsett M, et al: Human epidermal growth factor receptor 2 testing in breast cancer: ASCO-college of American pathologists guideline update. J Clin Oncol 41:3867-3872, 2023
26. Diéras V, Deluche E, Lusque A, et al: Trastuzumab deruxtecan (T-DXd) for advanced breast cancer patients (ABC), regardless HER2 status: A phase II study with biomarkers analysis (DAISY). Cancer Res 82, 2022 (suppl 4; abstr PD8-02)
27. Curigliano G, Hu X, Dent R, et al: Trastuzumab deruxtecan (T-DXd) vs physician's choice of chemotherapy (TPC) in patients (pts) with hormone receptor-positive (HR+), human epidermal growth factor receptor 2 (HER2)-low or HER2-ultralow mBC with Prior ET: Primary results from DESTINY-Breast06 (DB-06). J Clin Oncol 42, 2024 (suppl 17; abstr LBA1000)