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Discordance between Immunohistochemistry and Erb-B2 Receptor Tyrosine Kinase 2 mRNA to Determine Human Epidermal Growth Factor Receptor 2 Low Status for Breast Cancer

Keying Xu,* Jane Bayani,*† Elizabeth Mallon,‡ Gregory R. Pond,§ Tammy Piper,¶ Annette Hasenburg,‖ Christos J. Markopoulos,** Luc Dirix,†† Caroline M. Seynaeve,‡‡ Cornelis J.H. van de Velde,§§ Daniel W. Rea,¶¶ and John M.S. Bartlett*†¶

From Diagnostic Development, Ontario Institute for Cancer Research, Toronto, Ontario, Canada; the Laboratory Medicine and Pathobiology,† University of Toronto, Toronto, Ontario, Canada; the Department of Pathology,‡ Glasgow, United Kingdom; the Department of Oncology,§ McMaster University, Hamilton, Ontario, Canada; the Cancer Research UK Edinburgh Centre,¶ Institute of Genetics and Cancer, The University of Edinburgh, Edinburgh, United Kingdom; the Department of Gynecology and Obstetrics,‖ University Center Mainz, Mainz, Germany; the National and Kapodistrian University of Athens Medical School,** Athens, Greece; the Oncology Center,†† St. Augustinus Hospital, Antwerp, Belgium; the Department of Medical Oncology,‡‡ Erasmus MC Cancer Institute, Rotterdam, the Netherlands; the Department of Surgical Oncology,§§ Leiden University Medical Center, Leiden, the Netherlands; and the Cancer Research UK Clinical Trials Unit,¶¶ University of Birmingham, Birmingham, United Kingdom*

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Address correspondence to John M.S. Bartlett, B.Sc., Ph.D., FRCPath, Cancer Research UK Edinburgh Centre, Institute of Genetics and Cancer, The University of Edinburgh, Edinburgh, United Kingdom.
E-mail: john.bartlett@ed.ac.uk.

Novel human epidermal growth factor receptor 2 (HER2)-directed antibody-drug conjugates have demonstrated efficacy in HER2-low expressing breast cancers, which are currently defined as those with immunohistochemistry (IHC) scores of 1+ or 2+ with a negative *in situ* hybridization assay. However, current HER2 testing methods are designed to identify *HER2*-amplified tumors with high expression levels. The true definition of HER2-low expressing breast cancers remains controversial. Using quantitative molecular analysis of breast cancers based on RNA expression, the dynamic range of HER2 expression exceeds that detected by *in situ* IHC approaches. Erb-B2 receptor tyrosine kinase 2 (ERBB2) mRNA expression levels across IHC groups using patient samples derived from the Tamoxifen Exemestane Adjuvant Multicenter Trial were investigated. The standardized mean differences in ERBB2 mRNA scores in log base 2 are 0.47 (95% CI, 0.36−0.57), 0.58 (95% CI, 0.26−0.70), and 0.32 (95% CI, −0.12 to 0.75) when comparing IHC 0+ without staining versus IHC 0+ with some staining, IHC 0+ with some staining versus IHC 1+, and IHC 1+ versus IHC 2+/fluorescence *in situ* hybridization −negative, respectively. The results showed immunohistochemical methods have a comparatively limited dynamic range for measuring HER2 protein expression. The range of expression based on RNA abundance suggests a molecular method defining HER2-low cancers may better serve the treatment decision needs of this group. Indeed, the validity of RNA abundance to identify HER2-low cancers and predict treatment response needs to be further evaluated by prospective clinical trials. *(J Mol Diagn 2022, 24: 775−783; https://doi.org/10.1016/j.jmoldx.2022.04.002)*

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Current address of J.M.S.B., Cancer Research UK Edinburgh Centre, Edinburgh, UK.

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With the advent of anti−human epidermal growth factor receptor 2 (HER2) therapies, women with HER2-positive breast cancer now experience improved outcomes.[1] According to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines, HER2 positivity is assessed by a score of 3+ by immunohistochemistry (IHC) or gene amplification by *in situ* hybridization (ISH)−based techniques.[2,3] For 2+ IHC scores, reflex ISH testing is required to define HER2 status.[2,3] Across all breast cancers, approximately 13% to 15% are HER2 positive and 85% to 87% are HER2 negative when assessed using ASCO/CAP guidelines.[1,4] However, recently, a novel entity has been recognized, representing a subgroup of ASCO/CAP HER2-negative cancers. Currently described as HER2-low expressing tumors, interest in this grouping of breast cancers has been growing because of the development of novel treatment approaches targeting HER2. Two recent HER2-directed antibody-drug conjugates, trastuzumab deruxtecan and trastuzumab duocarmazine (SYD985), have demonstrated efficacy in HER2-low expressing breast cancers.[5−7] Should this approach prove effective in further trials, a large group of breast cancer patients may benefit from this new treatment modality. This raises the critical question of whether the classification of HER2 groups based on IHC and ISH, developed for the selection of patients with HER2 overexpression or gene amplification, is equally applicable to select patients with HER2-low expressing tumors. The current pragmatic definition of HER2-low cancers is those with a HER2 IHC score of 1+ or 2+ with negative ISH assay, and data suggest these cases form a group that comprises 45% to 55% of all breast cancers.[8] However, we and others[9,10] have demonstrated, using molecular analysis of breast cancers based on RNA expression, extremely broad ranges for mRNA expression for genes such as HER2, exceeding the dynamic range of conventional approaches, such as IHC.

Indeed, Erb-B2 receptor tyrosine kinase 2 (ERBB2) mRNA levels alone were recently found to be associated with pathologic response in both early-stage and metastatic settings, with patients possessing high levels of ERBB2 mRNA tending to experience better response to HER2-targeted therapies than those with low levels.[11,12] This suggests that, even within the HER2-positive group of tumors, mRNA expression may further refine prognostic or predictive value of IHC/ISH approaches. Prat et al[13] demonstrated that ERBB2 mRNA levels and HER2-enriched subtype provided independent and complementary information to identify tumors responsive to HER2-target therapy in HER2-positive

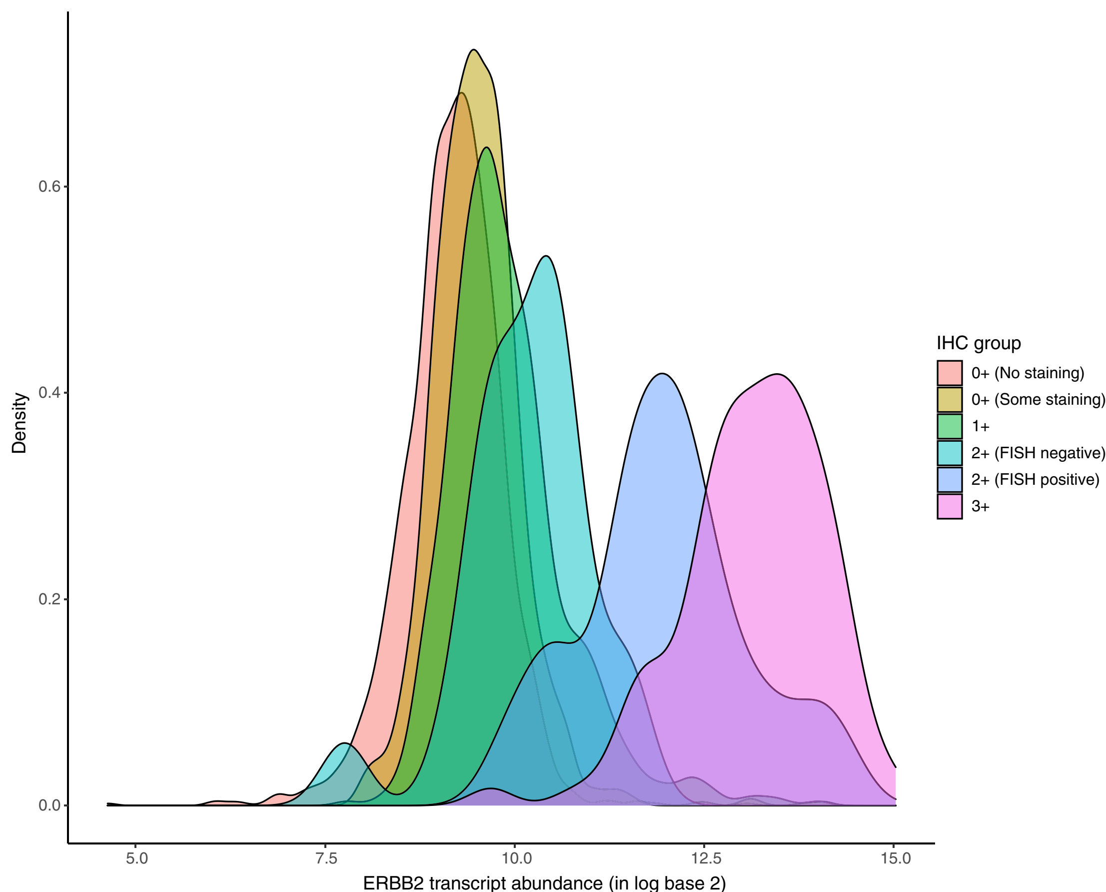

Figure 1 Smoothed histograms of ERBB2 transcript abundance (in log base 2) across immunohistochemistry (IHC) groups. Patient subgroups are defined as IHC 0+ without staining, IHC 0+ with some staining, IHC 1+, IHC 2+/fluorescence *in situ* hybridization (FISH) negative, IHC 2+/FISH positive, and IHC 3+.

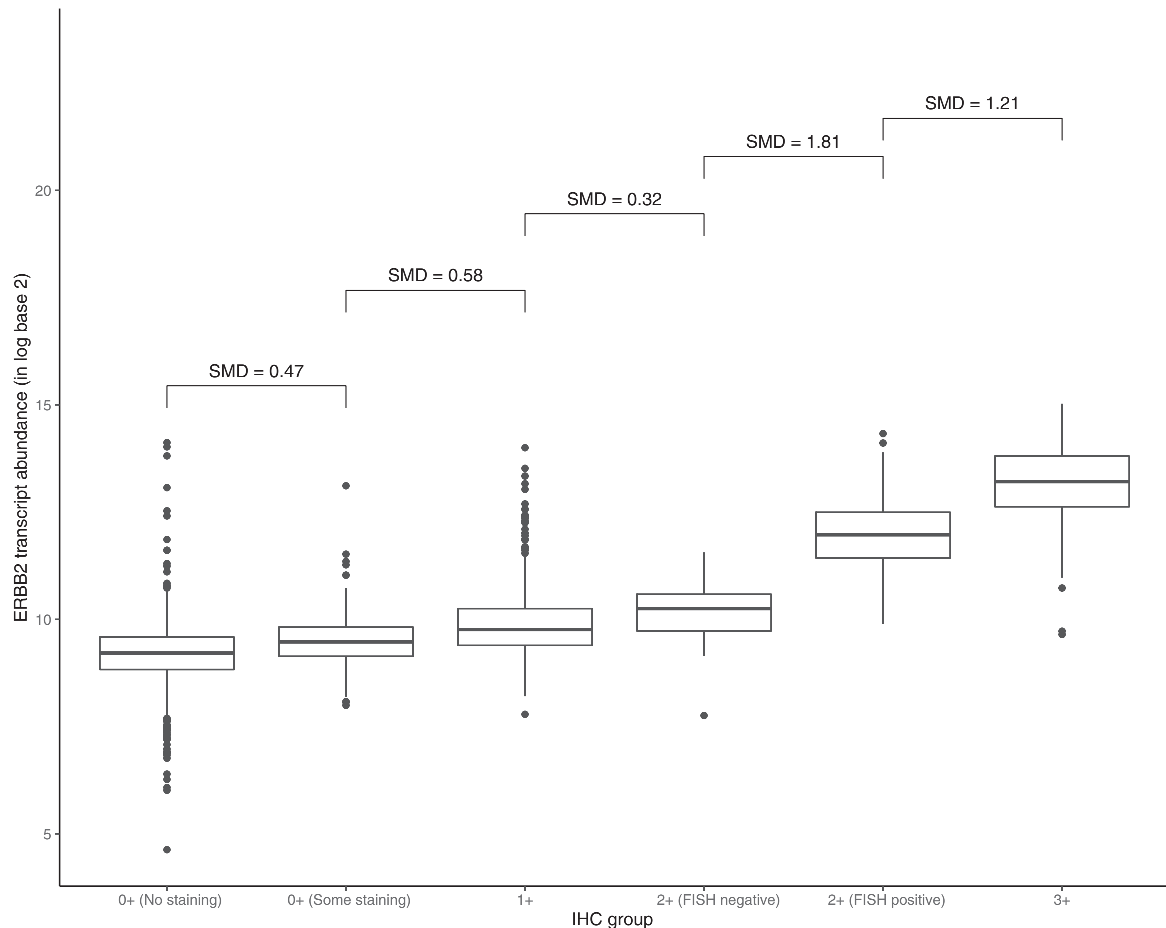

Figure 2 Boxplots of ERBB2 transcript abundance (in log base 2) across immunohistochemistry (IHC) groups. Patient subgroups are defined as IHC 0+ with no staining, IHC 0+ with some staining, IHC 1+, IHC 2+/fluorescence *in situ* hybridization (FISH) negative, IHC 2+/FISH positive, and IHC 3+. Standardized mean difference (SMD) of 0.2 represents a small effect size, 0.5 represents a medium effect size, and 0.8 represents a large effect size.

patients. In addition, in a recent randomized phase IIb trial of HER-targeted vaccine nelipepimut-S combined with trastuzumab as adjuvant treatment, HER2-negative tumors (according to ASCO/CAP guidelines) with high ERBB2 mRNA levels were reported to derive some benefit from this novel HER2-targeted vaccine.[14] In light of these and other studies, and to drive precision medicine to improve the clinical management of breast cancers, the relationship between HER2 expression by IHC and gene expression using the patient samples derived from the Tamoxifen Exemestane Adjuvant Multicenter (TEAM) Trial pathology study[15,16] was investigated, in an attempt to better define HER2-low cancers in the context of current diagnostic testing and how this may be improved.

Materials and Methods

Patient Data Set

IHC data[17] and NanoString gene expression[9] for HER2 from the TEAM Trial pathology study (Supplemental Table S1), [NCT00279448/NCT00032136 (*https://www.clinicaltrials.gov*, last accessed April 21, 2022), with Netherlands Trial Register Number NTR267 (*https://www.trialregister.nl*, last accessed April 21, 2022), and UMIN C000000057 (*https://www.umin.ac.jp/ctr*, last accessed April 21, 2022)][15,16] were used in this study. Only patients possessing complete information for HER2 IHC, mRNA levels, and fluorescence *in situ* hybridization (FISH) status were included for the analysis. HER2 IHC was performed on tissue microarrays in triplicate using the Hercept Test (Dako, Santa Clara, CA),[17] and FISH was performed as per ASCO/CAP guidelines using a commercial HER2/CEP17 assay,[18] and both were centrally assessed. RNA was extracted from the same formalin-fixed embedded tissue block from which the tissue core was obtained. The conventional IHC and quantitative mRNA expression were used to explore the range of HER2 expression in six patient subgroups based on their IHC scores, FISH status, and staining status. Namely, IHC 0+ with no staining observed, IHC 0+ where membrane staining is incomplete and is faint/barely perceptible and in ≤10% of tumor cells, IHC 1+ (incomplete membrane staining that is faint/barely perceptible and in >10% of

Table 1 Pairwise SMDs in ERBB2 mRNA Scores in Log Base 2 Comparing between IHC/FISH Groups

Variable	0+ (No staining)	0+ (Some staining)	1+	2+/FISH negative	2+/FISH positive	3+
0+ (No staining)	—	0.47 (0.36 to 0.57)	1.01 (0.91 to 1.10)	1.42 (0.98 to 1.85)	4.01 (3.64 to 4.37)	5.55 (5.31 to 5.79)
0+ (Some staining)		—	0.58 (0.46 to 0.70)	1.15 (0.71 to 1.59)	4.04 (3.60 to 4.47)	5.33 (4.98 to 5.67)
1+			—	0.32 (−0.12 to 0.75)	2.54 (2.17 to 2.91)	3.90 (3.64 to 4.16)
2+/FISH negative				—	1.81 (1.16 to 2.44)	3.22 (2.66 to 3.78)
2+/FISH positive					—	1.21 (0.81 to 1.59)
3+						—

Data are given as SMD (95% CI). Patient groups were defined by IHC scores and FISH status. Cohen suggested that an SMD of 0.2 represents a small effect size, 0.5 represents a medium effect size, and 0.8 represents a large effect size.

FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry; SMD, standardized mean difference.

tumor cells), IHC 2+ (weak to moderate complete membrane staining observed in >10% of tumor cells)/FISH-negative, IHC 2+ (weak to moderate complete membrane staining observed in >10% of tumor cells)/FISH-positive, and IHC 3+ (circumferential membrane staining that is complete, intense, and in >10% of tumor cells) expressing breast cancers, including those with *HER2* gene amplification.

Statistical Analysis

Standardized mean difference (SMD) was used in effect size analysis to indicate the standardized difference between two means of ERBB2 mRNA levels (in log base 2), along with 95% two-sided CIs. SMD can be calculated as the difference between the means divided by the pooled SD. SMD is also known as the Cohen *d*. Cohen suggested

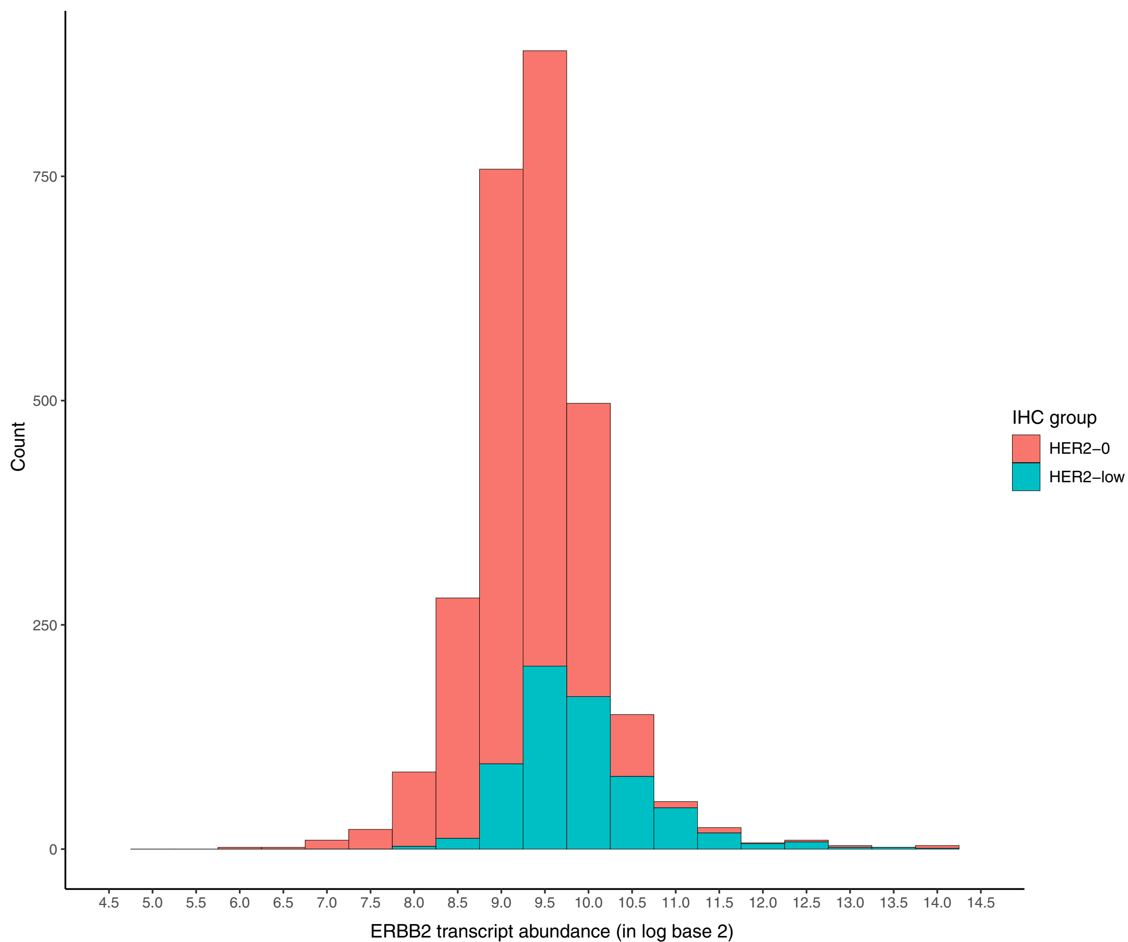

Figure 3 Distribution of immunohistochemistry (IHC) groups across ERBB2 mRNA levels for HER2-negative patients. Histograms of IHC HER2-low status across ERBB2 transcript abundance (in log base 2) with a bin width of 0.5. Patient subgroups are defined as HER2-0 and HER2-low. HER2-0 cancers were defined as those with IHC score of 0+. HER2-low cancers were defined as those with an IHC score of 1+ or 2+ with negative *in situ* hybridization assay.

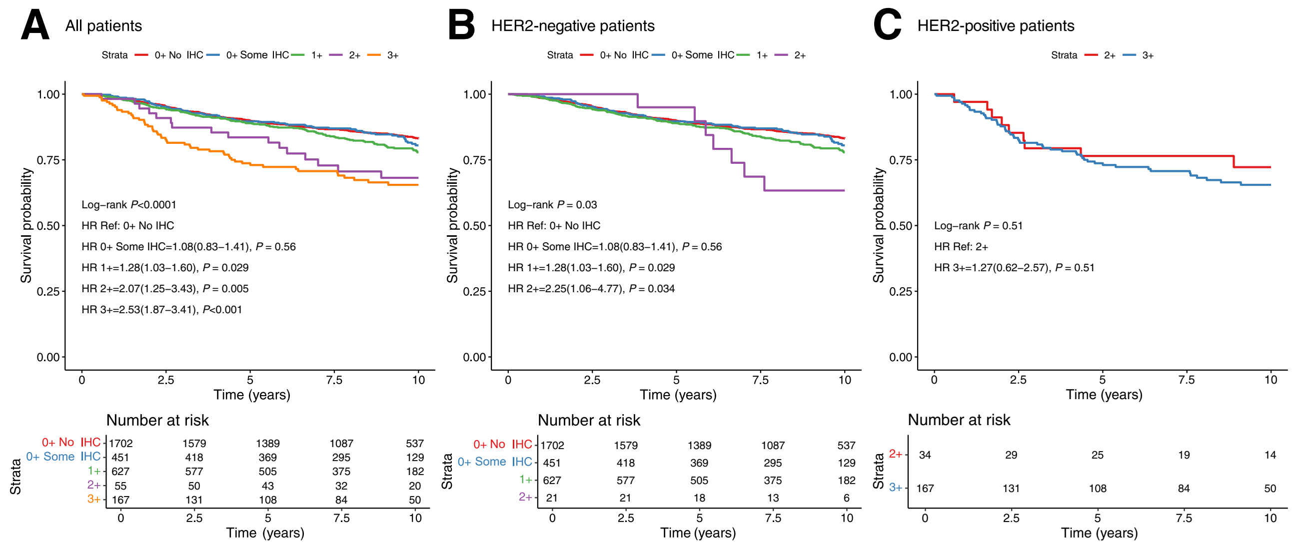

Figure 4 Prognosis performance of immunohistochemistry (IHC) scores. Kaplan-Meier survival curves with log-rank *P* values and hazard ratios (HRs) for breast cancer patients from the entire Tamoxifen Exemestane Adjuvant Multicenter Trial cohort (**A**), HER2-negative cases (**B**), and HER2-positive cases (**C**). In these panels, survival curves for IHC 0+ cases without staining, IHC 0+ cases with some staining, IHC 1+ cases, IHC 2+ cases, and IHC 3+ cases are plotted with hazard ratios calculated against IHC 0+ cases without staining (**A** and **B**) and against IHC 2+ cases (**C**). The 95% CIs for hazard ratios are in parentheses. For each group, the number at risk is presented under the *x* axis. Ref, reference.

that SMD of 0.2 represents a small effect size, 0.5 represents a medium effect size, and 0.8 represents a large effect size. Therefore, if the means of the two groups do not differ by ≥0.2 SDs, the difference between them is trivial, even if it is statistically significant.[19] The distant metastasis-free survival (DMFS) distributions were estimated using Kaplan-Meier method, and the log-rank test was used to assess the difference in survival distributions across groups. Hazard ratios (HRs) were calculated using the Cox proportional hazard model.[20] The assumptions of the Cox models were assessed by visual inspection of the Kaplan-Meier curves. The log relative hazards were estimated using natural cubic spline with three knots. Analyses were performed using in R 4.0.2 (*http://www.R-project.org*, last accessed February 17, 2022). The *rms* package in R 4.0.2 (*https://cran.r-project.org/web/packages/rms/index.html*,

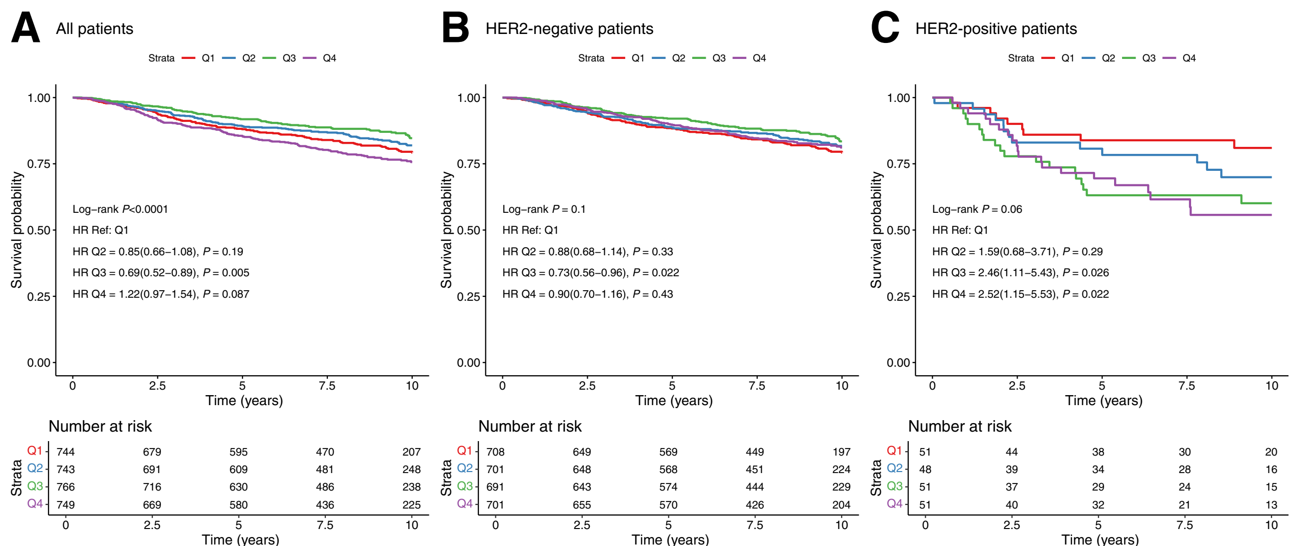

Figure 5 Prognosis performance of ERBB2 mRNA levels. Kaplan-Meier survival curves with log-rank *P* values and hazard ratios (HRs) for breast cancer patients from the entire Tamoxifen Exemestane Adjuvant Multicenter Trial cohort (**A**), HER2-negative cases (**B**), and HER2-positive cases (**C**). Within each panel, survival curves for subgroups defined by ERBB2 quantiles are plotted with hazard ratios calculated against cases in the first quantile (Q). Quantiles of ERBB2 mRNA levels are as follows: Q1, 4.63 to 9.00; Q2, 9.00 to 9.43; Q3, 9.43 to 9.90; and Q4, 9.90 to 15.03 for all patients; Q1, 4.63 to 8.98; Q2, 8.98 to 9.39; Q3, 9.39 to 9.78; and Q4, 9.78 to 14.12 for HER2-negative patients; and Q1, 9.65 to 12.33; Q2, 12.33 to 13.02; Q3, 13.02 to 13.72; and Q4, 13.72 to 15.03 for HER2-positive patients. The 95% CIs for hazard ratios are in parentheses. For each group, the number at risk is presented under the *x* axis. Ref, reference.

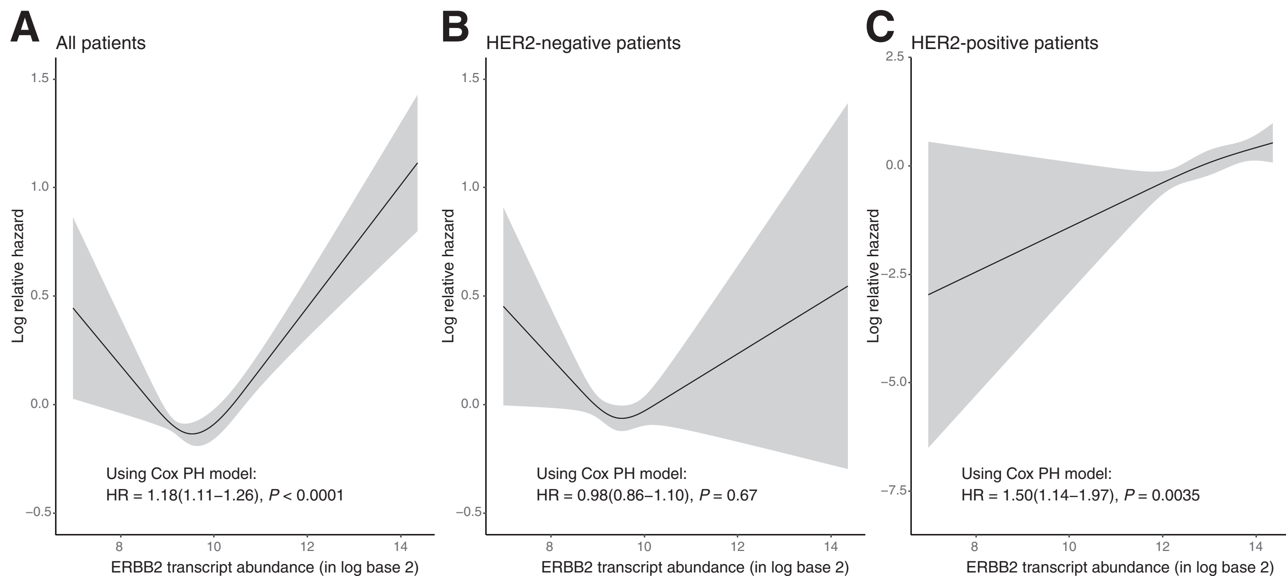

Figure 6 Log relative hazard curves of distant metastasis-free survival by continuous ERBB2 mRNA levels. Log relative hazard curves of distance metastasis-free survival by continuous ERBB2 mRNA levels with hazard ratios (HRs) and Wald *P* values for breast cancer patients from the entire Tamoxifen Exemestane Adjuvant Multicenter Trial cohort (**A**), HER2-negative cases (**B**), and HER2-positive cases (**C**). For each group, the **solid line** represents the average log hazard, and the **shaded area** is the 95% CI. Log relative hazard curves were based on restricted cubic spline with three knots. The 95% CIs for hazard ratios are in parentheses. PH, proportional hazard.

last accessed February 17, 2022) was used to calculate log relative hazards.

Results

Discordance between IHC Scores and ERBB2 mRNA Levels

A total of 3002 patients from the TEAM Trial pathology study were analyzed. Among the 3002 patients, 5.6% were identified as IHC 3+ ($n = 167$), 0.7% were identified as IHC 2+/FISH negative ($n = 21$), 1.1% were identified as IHC 2+/FISH positive ($n = 34$), 20.9% were identified as IHC 1+ ($n = 627$), 15.0% were identified as IHC 0+ with some staining ($n = 451$), and 56.7% were identified as IHC 0+ with no staining ($n = 1702$).

When the continuous distribution of ERBB2 mRNA scores in log base 2 was plotted across the six subgroups (IHC 0+ with no staining, IHC 0+ with some staining, IHC 1+, IHC 2+/FISH negative, IHC2+/FISH positive, and IHC 3+), the IHC 3+ group demonstrated the highest ERBB2 mRNA levels, with the distribution relatively distinct from the IHC2+/FISH-negative, IHC 1+, and IHC 0+ group, and moderate overlap with IHC 2+/FISH-positive group. In contrast, the histograms for the two IHC 0+ groups, IHC 1+ group, and IHC 2+/FISH-negative group showed significant overlaps, suggesting this group of patients share similar levels of ERBB2 mRNA, and are almost indistinguishable in terms of ERBB2 mRNA levels (Figure 1). Differences in mean levels of ERBB2 mRNA scores in log base 2 between any two adjacent IHC groups were statistically significant (*t*-test $P < 0.0001$). However, the statistical significance is partly driven by the large sample size; thus, *P* values can be misleading. Therefore, accounting for effect size allows us to remove the impact of large sample size; the SMDs in ERBB2 mRNA scores in log base 2 are 0.47 (95% CI, 0.36−0.57), 0.58 (95% CI, 0.26−0.70), and 0.32 (95% CI, −0.12 to 0.75) comparing IHC 0+ without staining versus IHC 0+ with some staining, IHC 0+ with some staining versus IHC 1+, and IHC 1+ versus IHC 2+/FISH negative, respectively (Figure 2 and Table 1). These illustrate that small or medium SMDs indicated that the ERBB2 mRNA levels were indistinguishable across different IHC groups. Therefore, patients with higher IHC scores did not show significantly greater ERBB2 mRNA mean levels than patients with lower IHC scores.

The distribution of HER2-low status was also plotted across ERBB2 mRNA intervals within HER2-negative patients (Figure 3). HER2-low cancers were defined as those with an IHC score of 1+ or 2+ with negative ISH assay. HER2-0 cancers were defined as those with IHC score of 0+. In this way, the definition of HER2-low status was based on the IHC score and ISH assay. The ERBB2 mRNA levels (in log base 2) intervals were defined from 4.5 to 14.5, with the bin width of 0.5. There were 9.1% (47/516), 17.0% (151/887), 27.1% (197/726), 46.1% (131/284), 72.3% (60/83), and 24.0% (18/75) HER2-low cancers in patient subgroups with ERBB2 mRNA levels (in log base 2) in the range of 8.5 to 9.0, 9.0 to 9.5, 9.5 to 10.0, 10.0 to 10.5, 10.5 to 11.0, and >11.0, respectively.

Table 2 The 5- and 10-Year DMFS by IHC Groups

Variable	All (N = 3002)	HER2 negative (n = 2801)	HER2 positive (n = 201)
5-Year DMFS			
0+ (No staining)	1702, 89.9 (88.4 to 91.3)	1702, 89.9 (88.4 to 91.3)	
0+ (Some staining)	451, 89.7 (86.8 to 92.6)	451, 89.7 (86.8 to 92.6)	
1+	627, 89.0 (96.5 to 91.5)	627, 89.0 (96.5 to 91.5)	
2+	55, 83.6 (74.3 to 94.0)	21, 95.0 (85.9 to 100)	34, 76.5 (63.5 to 92.1)
3+	167, 73.7 (67.2 to 80.9)		167, 73.7 (67.2 to 80.9)
10-Year DMFS			
0+ (No staining)	1702, 83.1 (81.1 to 85.1)	1702, 83.1 (81.1 to 85.1)	
0+ (Some staining)	451, 80.5 (76.3 to 84.9)	451, 80.5 (76.3 to 84.9)	
1+	627, 77.9 (74.2 to 81.7)	627, 77.9 (74.2 to 81.7)	
2+	55, 68.2 (56.2 to 82.6)	21, 63.3 (45.0 to 89.1)	34, 72.2 (58.1 to 89.8)
3+	167, 65.5 (58.2 to 73.7)		167, 65.5 (58.2 to 73.7)

Data are given as number, DMFS (95% CI). Patient groups were defined by IHC scores.
DMFS, distant metastasis-free survival; IHC, immunohistochemistry.

Impact of IHC Scores and ERBB2 mRNA Levels on Prognosis

An exploratory DMFS analysis was conducted across three clinical groups within the TEAM Trial (all patients, n = 3002; HER2-negative patients, n = 2801; HER2-positive patients, n = 201). DMFS was defined from the date of randomization. The median follow-up for all patients was 9.38 years (95% CI, 9.27−9.53 years). As described in *Materials and Methods*, the TEAM cohort is an anti-HER2 treatment naïve cohort.

In all patients (Figure 4A), DMFS estimates were statistically significantly different across the five IHC groups (log-rank $P < 0.0001$). Expectedly, IHC 3+ had the highest hazard ratio compared with IHC 0+/no staining (HR = 2.45; 95% CI, 1.82−3.30; $P < 0.0001$), followed by IHC 2+ (HR = 2.16; 95% CI, 1.32−3.52; $P = 0.002$) and IHC 1+ (HR = 1.27; 95% CI, 1.02−1.59; $P = 0.031$). No statistically significant difference was observed between IHC 0+/no staining and IHC 0+/some staining (HR = 1.06; 95% CI, 0.81−1.37; $P = 0.69$). In HER2-negative patients (Figure 4B), DMFS estimates were statistically significantly different across the four IHC groups (log-rank $P = 0.04$). Similarly, IHC 2+ had the highest hazard ratio compared with IHC 0+/no staining (HR = 2.21; 95% CI, 1.04−4.68; $P = 0.038$), followed by IHC 1+ (HR = 1.27; 95% CI, 1.02−1.59; $P = 0.031$). No statistically significant difference was observed between IHC 0+/no staining and IHC 0+/some staining (HR = 1.06; 95% CI, 0.81−1.37; $P = 0.68$). In HER2-positive patients (Figure 4C), no statistically significant difference was observed between IHC 2+ and IHC 3+ groups (log-rank $P = 0.70$).

When the four subgroups were defined by quartiles of ERBB2 mRNA levels, the DMFS scores in all patients (Figure 5A) were statistically significantly different across the four ERBB2 groups (log-rank $P < 0.0001$). The second quartile tended to have a lower hazard ratio compared with the first quartile (HR = 0.85; 95% CI, 0.66−1.08; $P = 0.18$). The third quartile had a significantly lower hazard ratio compared with the first quartile (HR = 0.71; 95% CI, 0.54−0.92; $P = 0.009$). The fourth quartile tended to have a higher hazard ratio compared with the first quartile (HR = 1.23; 95% CI, 0.98−1.33; $P = 0.08$). In HER2-negative patients (Figure 5B), no statistically significant

Table 3 The 5- and 10-Year DMFS by ERBB2 mRNA Quartile Groups

Variable	All (N = 3002)	HER2 negative (n = 2801)	HER2 positive (n = 201)
5-Year DMFS			
First quartile	744, 88.1 (85.7 to 90.5)	708, 88.3 (85.9 to 90.8)	51, 83.9 (74.2 to 94.8)
Second quartile	743, 89.2 (87.0 to 91.5)	689, 88.7 (86.3 to 91.1)	48, 80.7 (70.1 to 92.9)
Third quartile	766, 91.8 (89.9 to 93.8)	703, 92.0 (90.0 to 94.1)	51, 63.1 (50.9 to 78.3)
Fourth quartile	749, 85.4 (82.9 to 88.0)	701, 89.7 (87.4 to 92.0)	51, 69.5 (57.8 to 83.7)
10-Year DMFS			
First quartile	744, 79.2 (75.9 to 82.5)	708, 79.2 (75.8 to 82.6)	51, 81.0 (70.4 to 93.2)
Second quartile	743, 82.0 (78.9 to 85.1)	689, 81.6 (78.4 to 84.9)	48, 69.9 (57.3 to 85.3)
Third quartile	766, 84.7 (81.7 to 87.7)	703, 83.6 (80.5 to 86.9)	51, 60.1 (47.5 to 76.1)
Fourth quartile	749, 75.7 (72.3 to 79.2)	701, 81.1 (77.9 to 84.5)	51, 55.7 (42.6 to 72.9)

Data are given as number, DMFS (95% CI). Patient subgroups were defined by ERBB2 mRNA quartiles within each of the three cohorts (ie, all, HER2 negative, and HER2 positive).
DMFS, distant metastasis-free survival.

difference was observed across the four ERBB2 groups (log-rank $P = 0.2$). Similar to that, in all patients, only the third quartile had a significantly lower hazard ratio compared with the first quartile (HR = 0.71; 95% CI, 0.54−0.92; $P = 0.036$). Among HER2-positive patients (Figure 5C), no statistically significant difference was observed across the four ERBB2 groups (log-rank $P = 0.1$). Unlike all/HER2-negative patients, the third and fourth quartiles both had significantly higher hazard ratios compared with the first quartile (HR = 2.21; 95% CI, 1.03−4.67; $P = 0.042$; and HR = 2.28; 95% CI, 1.07−4.87; $P = 0.034$; respectively). The relationship between ERBB2 mRNA levels as a continuous variable and log relative hazards was then investigated. U-shaped curves were observed in all and HER2-negative patients. These two curves showed that the distant metastasis-free survival first became better as the ERBB2 mRNA levels increase and achieved the optimum for ERBB2 mRNA level (in log2 base) at around 9.5. Then, increased ERBB2 mRNA level was associated with worse distant metastasis-free survival (Figure 6, A and B). A linear relationship between ERBB2 mRNA levels and log relative hazards was observed in HER2-positive patients, which indicates that increased ERBB2 mRNA level was associated with worse distant metastasis-free survival (Figure 6C).

Discussion

This study shows that immunohistochemical methods have a comparatively limited dynamic range for measuring HER2 expression, especially in HER2-negative patients. The range of expression based on RNA abundance suggests that a molecular method defining HER2-low cancers may better serve the treatment decision needs of this group. However, the clinical validity of ERBB2 mRNA levels to identify HER2-low cancers and predict treatment response needs to be further evaluated by establishing defined cut points and validating in the context of retrospective and prospective clinical trials, specifically in the context of anti-HER2 therapy.

It was observed that patients in different IHC groups were indistinguishable in terms of ERBB2 mRNA levels, especially for those identified as HER2 negative (Figure 1). The ERBB2 mRNA levels (in log base 2) of HER2-low cancers ranges from 7.76 to 14.0. Patients with higher IHC scores did not show significantly greater ERBB2 mRNA mean levels than patients with lower IHC scores. These findings indicated that inconsistencies exist in determining HER2 expression levels of breast cancer patients using ERBB2 mRNA levels versus IHC scores. Prat et al[13] showed that PAM 50 subtypes HER2-E and ERBB2 mRNA provided independent and complementary information about responsiveness to HER2-targeted therapies. This might also be the case for IHC scores and ERBB2 mRNA levels in HER2-low expressing cancers, and this is worth exploring in further studies.

Because patients in the TEAM Trial were not treated by any anti-HER2 treatment, information about the responsiveness to HER2-targeted treatment was not available. The associations between IHC scores/ERBB2 mRNA levels and DMFS were explored in both HER2-negative and HER2-positive patients. In HER2-negative patients, patients with higher IHC scores showed a worse survival outcome (Figure 4). For example, a hazard ratio of 1.28 (95% CI, 1.08−1.60) was observed for those defined as IHC 1+ compared with IHC 0+/without staining. In contrast, the U-shaped relationship between continuous ERBB2 mRNA levels and hazard ratios seen (Figure 6) supports previous observations.[21] Interestingly, the survival curves separated only after about 5 years in HER2 nonamplified cases. No association was observed between 5-year survival and HER2-low expression assessed by either IHC or ERBB2 mRNA (Tables 2 and 3). In HER2-positive patients, there is no significant difference in DMFS between the IHC 2+/FISH-positive and IHC 3+ group. However, a linear relationship between continuous ERBB2 mRNA levels and hazard ratios was observed. That is, the higher the ERBB2 mRNA levels, the worse the survival outcome.

This study represents a large phase III clinical trial cohort of patients, not treated with HER2 targeted therapies, with extensive follow-up. It was demonstrated that in the conventional HER2-positive group (IHC 3+/FISH amplified), mRNA expression levels can provide additional prognostic information, consistent with previous data regarding HER2-driven cancers using multigene testing.[13] Conversely, in the HER2-negative group, both central IHC performed in this study and mRNA expression appeared to provide prognostic information. However, for mRNA, the impact on prognosis appears to be bimodal, as seen previously.[21] This may represent a mixed effect of HER2 expression (in the higher-expressing patients) and/or a potential link between low HER2 expression and basal-like features.

In a recently published retrospective study investigating the features of HER2-low breast cancers,[22] overlap of ERBB2 mRNA levels among IHC groups was observed in both triple-negative and hormone receptor−positive breast cancers. Therefore, it may be important to take hormone receptor status into consideration when developing new methods for identifying eligible HER2-targetable patients. Recently, it was demonstrated that ERBB2 mRNA levels might provide a better selection of patients who benefit from the antibody-drug conjugate T-DM1,[12] where higher ERBB2 levels are suggestive of better response rates to HER2-targeted treatment.

There remains considerable controversy about the true parameters of an HER2-low expressing group of breast cancers. How to use ERBB2 levels to identify HER2-targeted groups in HER2-negative patients will need further investigation and robust assays.

Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.jmoldx.2022.04.002*.

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