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ORIGINAL REPORT

HER2 and Chromosome 17 Effect on Patient Outcome in the N9831 Adjuvant Trastuzumab Trial

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ABSTRACT

Purpose
We examined associations between tumor characteristics (human epidermal growth factor receptor 2 [HER2] protein expression, *HER2* gene and chromosome 17 copy number, hormone receptor status) and disease-free survival (DFS) of patients in the N9831 adjuvant trastuzumab trial.

Patients and Methods
All patients (N = 1,888) underwent chemotherapy with doxorubicin and cyclophosphamide, followed by weekly paclitaxel with or without concurrent trastuzumab. HER2 status was determined by immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) at a central laboratory, Mayo Clinic, Rochester, MN. Patients with conflicting local positive HER2 expression results but normal central laboratory testing were included in the analyses (n = 103).

Results
Patients with HER2-positive tumors (IHC 3+, FISH *HER2*/centromere 17 ratio ≥ 2.0, or both) benefited from trastuzumab, with hazard ratios (HRs) of 0.46, 0.49, and 0.45, respectively (all $P < .0001$). Patients with *HER2*-amplified tumors with polysomic (p17) or normal (n17) chromosome 17 copy number also benefited from trastuzumab, with HRs of 0.52 and 0.37, respectively ($P < .006$). Patients who received chemotherapy alone and had *HER2*-amplified and p17 tumors had a longer DFS than those who had n17 (78% *v* 68%; $P = .04$), irrespective of hormone receptor status or tumor grade. Patients with HER2-normal tumors by central testing (n = 103) seemed to benefit from trastuzumab, but the difference was not statistically significant (HR, 0.51; $P = .14$). Patients with hormone receptor–positive or –negative tumors benefited from the addition of trastuzumab, with HRs of 0.42 ($P = .005$) and 0.60 ($P = .0001$), respectively.

Conclusion
These results confirm that IHC or FISH HER2 testing is appropriate for patient selection for adjuvant trastuzumab therapy. Trastuzumab benefit seemed independent of *HER2*/centromere 17 ratio and chromosome 17 copy number.

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INTRODUCTION

Human epidermal growth factor receptor 2 (HER2) is involved in the pathophysiology of breast cancer (BC), and its protein expression and gene amplification status help predict outcome of patients with early and advanced disease. Development and use of effective therapies that target the HER2 protein (eg, trastuzumab, lapatinib) depend on accurate HER2 testing of tumor specimens to optimize patient selection.[1] However, HER2 testing remains controversial because of technical issues and laboratory experience, accuracy of testing and cut point determination, differential interpretation among pathologists, tumor heterogeneity, and preliminary clinical data suggesting that even patients with HER2-normal tumors (eg, immunohistochemistry [IHC] scores of 0 to 2+ and negative fluorescent in situ hybridization [FISH] results) may benefit from trastuzumab treatment.[2,3]

The successful conduct of our phase III, North Central Cancer Treatment Group–led N9831 trial with standardized HER2 testing and thorough patient follow-up allows us to critically examine the ability of HER2 protein expression and gene amplification to predict adjuvant trastuzumab benefit.

N9831 evaluated patients with resected HER2-positive BC treated with combination chemotherapy with or without trastuzumab.[4] Examination of our large N9831 HER2 data set in relation to standard clinicopathologic characteristics may result in more robust conclusions than those previously reached with data from small trials. Importantly, predicting benefit of anti-HER2 therapy may not be identical in the metastatic and adjuvant settings. There is speculation regarding whether HER2 protein overexpression or gene amplification is the better predictor of trastuzumab benefit. Chromosome 17 polysomy may be associated with trastuzumab response, particularly in patients with metastatic BC with *HER2*-nonamplified tumors.[5,6] Because HER2 is differentially expressed between HER2-positive/hormone receptor–positive and HER2-positive/hormone receptor–negative tumors,[7-15] hormone receptor status in relation to HER2 levels also may be important in predicting trastuzumab benefit.[16] Because HER2 protein levels, *HER2* gene copy number, chromosome 17 copy number, and hormone receptor status may influence trastuzumab response, we report their associations with disease-free survival (DFS) in 1,888 patients randomly assigned to receive chemotherapy with or without concurrent trastuzumab in N9831.

PATIENTS AND METHODS

Patients

The N9831 trial (Phase III Trial of Doxorubicin and Cyclophosphamide Followed by Weekly Paclitaxel With or Without Trastuzumab as Adjuvant Treatment for Women With HER-2 Over-Expressing or Amplified Node Positive or High Risk Node Negative Breast Cancer) was approved by participating institutional review boards. The study had three arms: arm A, doxorubicin and cyclophosphamide followed by weekly paclitaxel; arm B, same as arm A but followed by 1 year of sequential trastuzumab; arm C, same as arm A but with 1 year of concurrent trastuzumab, started the same day as paclitaxel. The present analyses included patients randomly assigned to arms A versus C, enrolled from May 25, 2000, through January 23, 2002, and from September 2, 2002, through April 25, 2005, and tested for HER2 protein overexpression or gene amplification at a central laboratory (Mayo Clinic, Rochester, MN). Outcome data of patients in arm B had not been released by the study's independent data monitoring committee at time of analysis and are not included in this report.

HER2 Testing Methods

IHC staining was performed on paraffin-embedded 5-μm tissue sections using the HercepTest according to the manufacturer's instructions (DAKO, Carpinteria, CA).[17-19] Assay control cell lines (SK-BR-3:3+, MDA-175:1+, MDA-231:0) provided on slides in the HercepTest kit were analyzed in each assay. Invasive carcinoma cells (and not benign epithelial or ductal carcinoma in situ cells) were used for the assessment of HER2 status of the tumor. Specimens were scored as per the instructions in the trastuzumab package insert.[20] A specimen with at least 10% invasive cells with complete membrane staining was classified as 3+ and considered HER2-positive according to pre–American Society of Clinical Oncology/College of American Pathologists 2007 guidelines.[1]

FISH analysis was performed on deparaffinized 5-μm tissue sections using the PathVysion *HER2* DNA probe kit and the *HER2*/centromere 17(*HER2*/centromere enumerator probe for chromosome 17 [CEP17]) probe mixture (Abbott Molecular, Des Plaines, IL).[17-19] For each case, a parallel hematoxylin and eosin–stained slide was examined for regions of invasive carcinoma by a board-certified pathologist (D.W.V., R.P.K.). The complete tissue section was scanned by two certified cytogenetic technologists to detect any subpopulation of amplified cells. Thirty representative nuclei from the invasive tumor were scored by each technologist (60 nuclei total), with an overall evaluation performed by a board-certified pathologist (R.P.K., R.B.J.). When the red *HER2* signals were clearly amplified (large clouds of amplification), we assigned ≥ 20 red signals and counted the green (CEP17) signals. For such cases, a number needs to be defined for the numerator and thus the ratio was defined as 20/average number of green signals per cell. As polysomy 17 increases, the ratio decreases. Scoring ranges were based on those determined for the US Food and Drug Administration–approved test for *HER2* gene alterations in BC.[21,22] A specimen with an *HER2*/CEP17 ratio ≥ 2.0 in invasive cells was classified as *HER2* amplified and considered *HER2* positive according to pre–American Society of Clinical Oncology/College of American Pathologists 2007 guidelines.[1]

Because many different *HER2* and chromosome 17 alterations have been observed in BC,[23-27] we independently categorized the *HER2* FISH results on the basis of *HER2* and CEP17 signal patterns. For *HER2*-amplified tumors (*HER2*/CEP17 ratio ≥ 2), three ranges of CEP17 signals were observed: polysomy 17 (p17), ≥ 3 CEP17 signals in more than 30% of nuclei; monosomy 17 (m17), 0 to 1 CEP17 signals in more than 60% of nuclei; and normal (n17) all other cases. We carefully validated these polysomy and monosomy cutoffs by extensively analyzing our N9831 data and a large set (> 10,000 cases) of clinical *HER2* FISH assays concurrently performed by the central testing laboratory (Data Supplement). Both cutoffs clearly distinguish chromosome 17 polysomic and monosomic cases from those cases without chromosome 17 centromere anomalies. All categorization thresholds were selected to reduce the rate of false-positive findings for gene amplification, gene deletion, and chromosome loss or gain. In our experience, these criteria have worked well to correct for truncation and nuclear overlap and the increase in four CEP17 signals due to G2M for nearly all solid tumors. Table 1 provides detailed definitions for *HER2* amplification, small clones of *HER2* amplification, *HER2* duplication, and chromosome 17 loss or gain.

Quality control of the *HER2* FISH test is assessed routinely according to standard College of American Pathologists and the American College of Medical Genetics guidelines.[28,29] The performance of the assay as assessed on a monthly basis has been stable according to Westgard rules.[30]

Eligibility criteria for N9831 trial enrollment were initially based on local laboratory HER2 test results (IHC score of 3+ or *HER2*/control probe ratio ≥ 2.0 or five or more gene copies of *HER2*).[18,19] After analysis of the first 119 specimens showed poor concordance between HER2 results from local and central (Mayo Clinic) laboratories,[19] the protocol was amended (amendment

Table 1. Classification of *HER2*/CEP17 Data

Criteria for Classifying Each Specimen
HER2
Amplified *HER2*: > 10 *HER2* signals in > 40% of invasive nuclei
Small clone of amplified *HER2*: > 10 *HER2* signals in > 5 and < 40% of invasive nuclei
Duplicated *HER2*: having an *HER2*/CEP17 ratio > 1.30, but not amplified *HER2*
Deleted *HER2* (–*HER2*): having an *HER2*/CEP17 ratio < 0.80
CEP17
Polysomic 17 (+17; p17): ≥ 3 CEP17 copies in > 30% of invasive nuclei
Monosomic 17 (–17; m17): 1 CEP17 copy in > 60% of invasive nuclei
The final interpretation combined the *HER2* and CEP17 results as follows:
NACA: Normal for all chromosome 17 anomalies (*HER2*/CEP17 ratio > 0.80 and < 1.30, < 30% nuclei with ≥ 3 CEP17 signals, < 60% nuclei with 1 CEP17 signal).
Normal *HER2*, −17: One CEP17 copy in > 60% of invasive nuclei and two *HER2* copies
Amplified *HER2*, +17: Amplified *HER2* and +CEP17.
Amplified *HER2*, −17: Amplified *HER2* and –CEP17.
+17: ≥ 3 *HER2* and CEP17 copies in > 30% of invasive component (ratio > 0.80 and < 1.30).
–17: 1 *HER2* and CEP17 copy in > 60% of invasive component (ratio > 0.80 and < 1.30).

Abbreviations: CEP17, centromere enumerator probe for chromosome 17; NACA, no apparent chromosome abnormality.

7), to require validation of HER2 positivity by the central laboratory for eligibility and study participation. When the central laboratory's IHC and FISH test results were both negative, the local site was contacted and another set of slides was submitted to a reference laboratory (Laboratory Corporation of America, Research Triangle Park, NC). Enrollment into N9831 was then allowed only if HER2 positivity could be confirmed by IHC or FISH performed in the central or reference laboratories.[18] One hundred three patients with HER2-normal tumors (as shown by central laboratory IHC and FISH test results) continued in the trial because of local laboratory positivity (90 patients enrolled before amendment 7 was established) or because of reference laboratory positivity (13 patients enrolled after amendment 7). In the present analyses, data from the central laboratory tests were used.

Statistical Methods

DFS was the primary end point, defined as local, regional, or distant recurrence, contralateral BC, another primary cancer (except squamous or basal cell carcinoma of the skin, carcinoma in situ of the cervix, or lobular carcinoma in situ of the breast), or death from any cause. The duration of DFS was defined as the time from registration to the first event. DFS was estimated by the Kaplan-Meier method. Comparisons between arms A and C were performed using the Cox proportional hazards model, stratified by nodal status (negative, 1 to 3+, 4 to 9+, 10+, and sentinel node positive) and hormone receptor status (estrogen receptor [ER] positive and/or progesterone receptor [PR] positive *v* negative for both receptors).

RESULTS

Study Patients

The trial enrolled 1,888 eligible patients into arms A and C; 1,795, 1,845, and 1,783 were evaluated for *HER2* gene amplification, HER2 protein overexpression, or both, respectively. We found small clones of *HER2* amplification (Table 1) in 4.5% of patient specimens. The clinicopathologic characteristics of the patients are described in the Data Supplement, and the clinicopathologic characteristics stratified by IHC and FISH status are described in Table 2.

HER2 Protein Expression, Gene/Chromosome 17 Ratio, and Gene Copy Number

A 54%, 51%, and 55% improvement in DFS was observed with the addition of trastuzumab for patients whose tumors had HER2 IHC scores of 3+ (Fig 1A), *HER2*/CEP17 ratios of ≥ 2.0 (Fig 1B), or both (Fig 1C), respectively. The hazard ratios (HRs) for patients with IHC scores of 3+, *HER2*/CEP17 ratios of ≥ 2.0, or both were 0.46, 0.49, and 0.45, respectively (Fig 2). Patients with IHC scores of 0 to 2+ (Figs 1D and 2A), *HER2*/CEP17 ratio less than 2.0 (Figs 1E and 2A), or both (Figs 1F and 2B) had HRs of 0.69, 0.54, and 0.51, respectively, with the addition of trastuzumab, but the differences were not statistically significant ($P = .26, .12$, and $.14$, respectively). Figure 2C shows that at least a 49% improvement in DFS was observed with the addition of trastuzumab for patients in the four *HER2*/CEP17 ratio subgroups (2 to 4.99, 5 to 7.99, 8 to 10.99, and 11 to 14.99). The subgroup with *HER2*/CEP17 ratio of ≥ 15.0 had an insufficient number of events to determine whether trastuzumab treatment was efficacious, but the HR was close to 1. Patients with *HER2* copy number of four or more received benefit from receiving trastuzumab (HR, 0.51; 95% CI, 0.37 to 0.70; $P < .0001$). Similarly, patients with *HER2* copy number less than four received benefit from receiving trastuzumab (HR, 0.52; 95% CI, 0.29 to 0.94; $P = .03$).

Table 2. Patient Characteristics for Patients by FISH/IHC Status (N = 1,783)

Characteristic	FISH < 2.0/IHC 0, 1, 2+ No.	%	FISH ≥ 2.0 or IHC 3+ No.	%	χ^2 *P*
Total patients	103	6	1,680	94	
Age, years					.42
< 40	16	16	272	16	
40-49	36	35	564	34	
50-59	39	38	549	33	
≥ 60	12	12	295	18	
Race					.82
White	86	84	1,417	84	
Other	17	17	263	16	
Menopausal status					.67
Premenopausal	57	55	893	53	
Postmenopausal	46	45	787	47	
Estrogen/progesterone status					< .0001
ER or PR positive	83	81	893	53	
Other	20	19	787	47	
Nodal status					.015
Node positive (1-3+ nodes)	52	50	649	39	
Node positive (4-9+ nodes)	27	26	416	25	
Node positive (10+ nodes)	15	15	216	13	
Node negative (no positive nodes)	2	2	88	5	
Positive sentinel node	5	5	140	8	
Negative sentinel node	2	2	171	10	
Surgery					.34*
Breast conserving	36	35	665	40	
Mastectomy	67	65	1,012	60	
Missing	0	0	3	0.2	
Predominant tumor histology					< .0001
Ductal	87	85	1,603	95	
Lobular	14	14	40	2	
Mucinous	0	0	7	0.4	
Papillary	1	1.0	3	0.2	
Medullary	1	1.0	5	0.3	
Tubular/cribriform	0	0	0	0	
Intraductal	0	0	3	0.2	
Other	0	0	19	1	
Histologic tumor grade (Elston/SBR)					< .0001
Well/intermediate	50	49	458	27	
Poor	53	51	1,222	73	
Pathologic tumor size, cm					.042
< 2	24	23	554	33	
≥ 2	79	77	1,126	67	
Received hormonal treatment					< .0001*
Yes	82	89	842	51	
No	20	20	816	49	
Missing	1	1	22	1	
Duplicated					< .0001
Yes	27	26	168	10	
No	76	74	1,512	90	
Chromosome 17					.042*
Polysomy	42	74	950	57	
Normal	13	23	619	37	
Monosomy	2	4	100	6	
ND	46		11		

NOTE. Associations between HER2-normal and HER2-positive patients were assessed using χ^2 tests. HER2-normal patients were more likely than HER2-positive patients to have hormone receptor–positive breast cancer($P < .0001$), have lobular carcinoma ($P < .0001$), have well/intermediate differentiated tumors ($P < .0001$), and duplicated *HER2* ($P < .0001$).

Abbreviations: FISH, fluorescent in situ hybridization; IHC, immunohistochemistry; ER, estrogen receptor; PR, progesterone receptor; ND, no data.

*Missing and ND values not included in analysis.

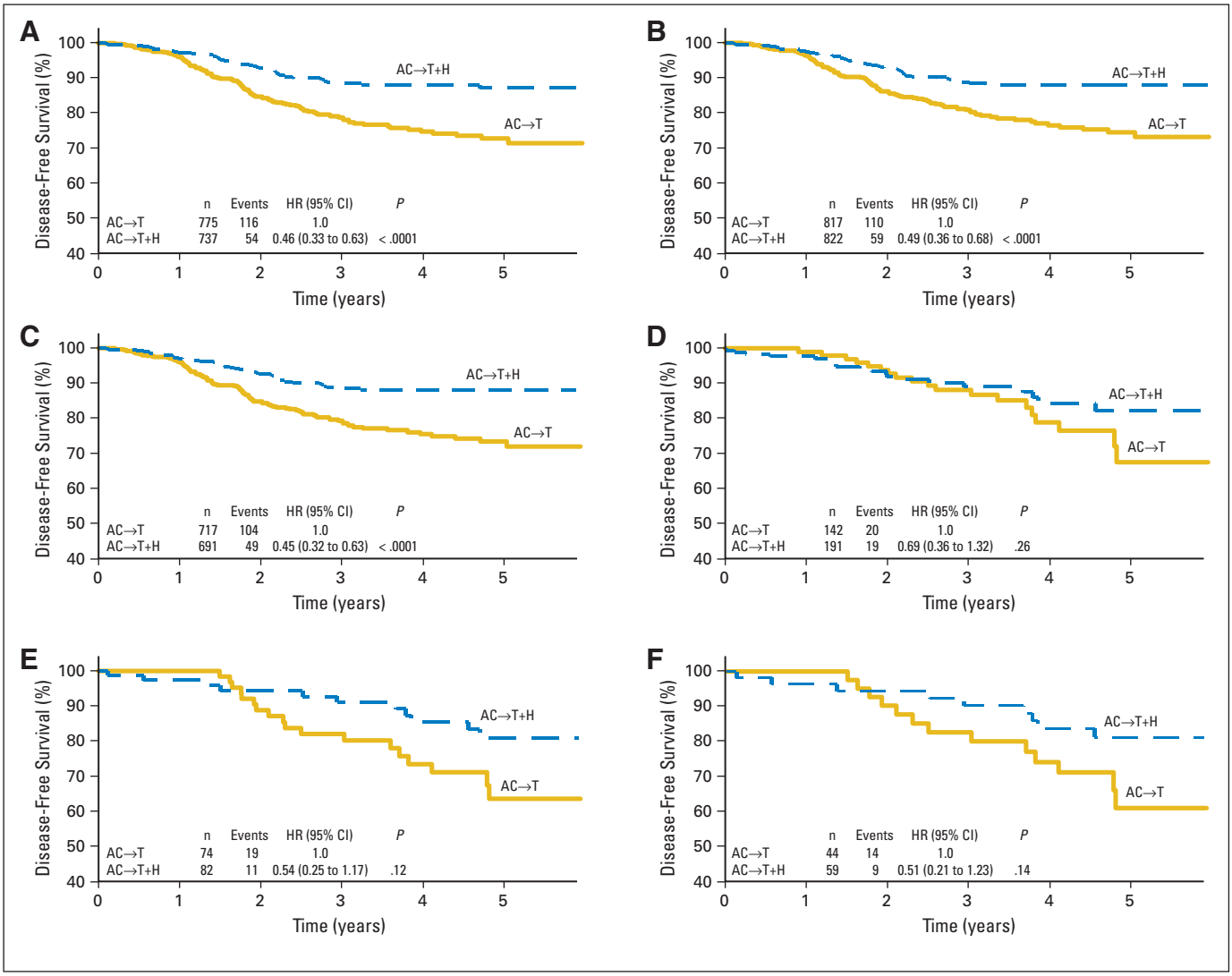

Fig 1. Kaplan-Meier curves for disease-free survival (DFS). Patients treated with doxorubicin, cyclophosphamide, and paclitaxel are shown by the solid gold line. Patients treated with doxorubicin, cyclophosphamide, paclitaxel, and trastuzumab are shown by the dashed blue line. (A) DFS of patients with human epidermal growth factor receptor 2 (HER2) protein overexpression (immunohistochemistry [IHC] score, 3+). (B) DFS of patients with *HER2* gene amplification (*HER2*/centromere enumerator probe for chromosome 17 [CEP17] ratio, ≥ 2.0). The *HER2*/CEP17ratio was determined by fluorescent in situ hybridization. (C) DFS of patients with HER2 protein overexpression and gene amplification (IHC score, 3+; *HER2*/CEP17 ratio, ≥ 2.0). (D) DFS of patients with normal HER2 protein expression (IHC score, 0 to 2+). (E) DFS of patients with normal *HER2* amplification (*HER2*/CEP17 ratio, < 2.0). (F) DFS of patients with normal HER2 protein expression and *HER2* nonamplification (IHC score, 0 to 2+; *HER2*/CEP17 ratio, < 2.0). AC, doxorubicin plus cyclophosphamide; T, paclitaxel; H, trastuzumab.

Chromosome 17 Copy Number Aberrations

The distribution of copy number anomalies was as follows: *HER2* amplified with n17, 29%; *HER2* amplified with p17, 46%; *HER2* amplified with m17, 4%; small clones of *HER2* amplification, 5%; *HER2* duplicated, 6%; *HER2* not amplified, 6%; and *HER2* not amplified with p17, 2% (n = 37; Fig 3A). Among the patients with *HER2* amplification, *HER2*/CEP17 ratios tended to range from 6.00 to 15.00 with n17 background, 3.0 to 10.99 with p17 background, 15.0 to 18.99 with m17 background, and 2.00 to 5.99 with small clones. Ratios tended to be less than 3.00 if duplicated. Figure 3B illustrates cells with *HER2* amplification with n17 and p17 backgrounds. Polysomy 17 was significantly associated with high Nottingham grade but not hormone receptor status. Among the 1635 patients with either p17 or n17, 75% of p17 had high-grade tumors, whereas 68% of n17 had high-grade tumors ($P = .002$; Data Supplemental).

Patients with *HER2* amplification and either p17 or n17 backgrounds had improved HRs (0.52 and 0.37, respectively) and similar 5-year DFS rates (89% and 88%, respectively) with addition of trastuzumab (Figs 4A and 4B). Conversely, among patients who received chemotherapy alone, those with p17 seemed to have longer DFS than those with n17 (Fig 4A; $P = .04$). In multivariate analysis, p17 was still significant ($P = .01$), with hormone receptor status, nodal status, and tumor size being significant covariates (Data Supplement). Within arm A, there was a trend that p17 was associated with high Nottingham grade ($P = .07$), but not with hormone receptor status ($P = .89$; Data Supplement).

Hormone Receptor Status

One thousand twenty-two patients (54%) had ER- and/or PR-positive tumors, and 866 patients (46%) had ER- and PR-negative

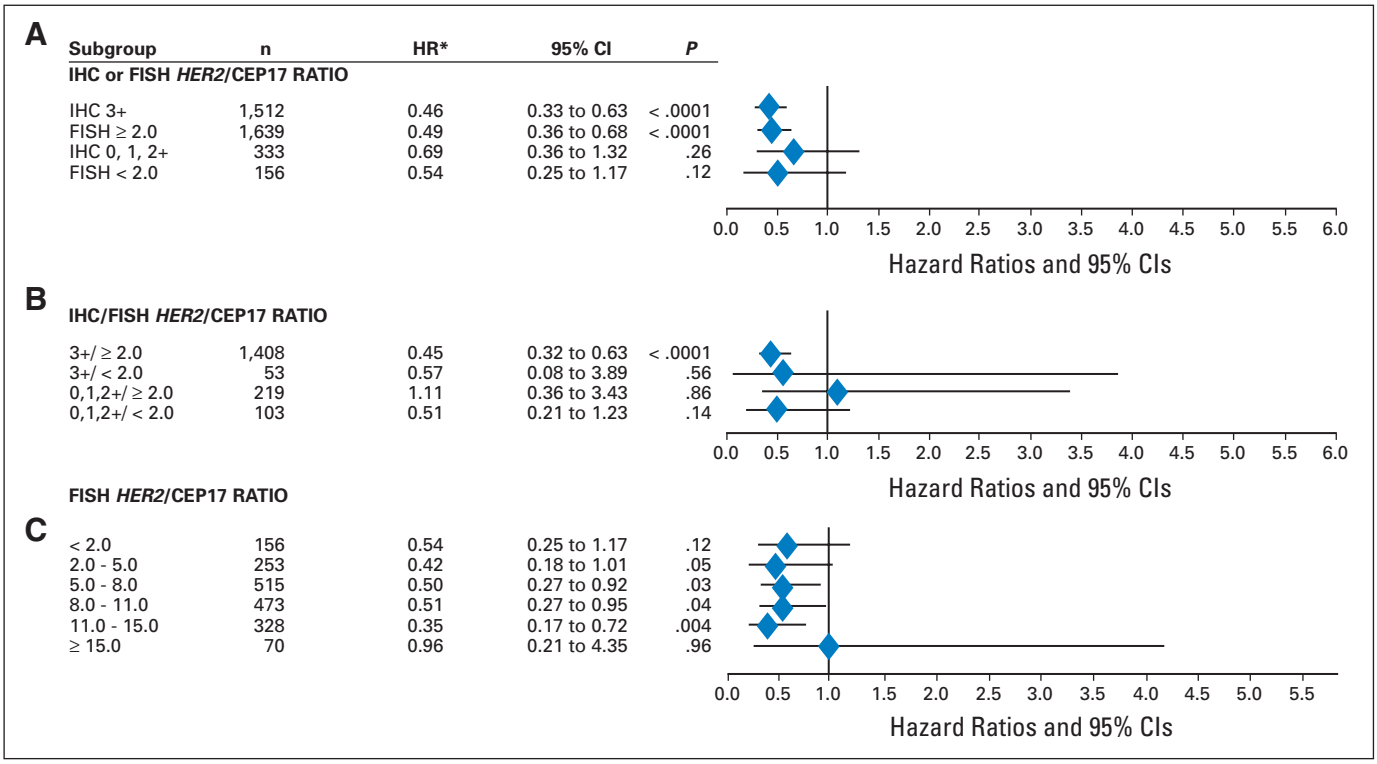

Fig 2. Hazard ratios by HER2 protein expression and gene amplification subgroups according to (A) immunohistochemistry (IHC) or fluorescent in situ hybridization (FISH); (B) IHC and FISH; and (C) FISH alone. (*) Disease-free survival hazard ratios calculated for comparing patients treated with and without trastuzumab. Comparisons for each subgroup were performed using Cox proportional hazard models stratified by nodal status and hormone receptor status. HR, hazard ratio; CEP17, centromere enumerator probe for chromosome 17.

tumors. Patients with ER/PR-positive or -negative tumors benefited from the addition of trastuzumab with HRs of 0.42 (95% CI, 0.27 to 0.65; $P = .0001$) and 0.60 (95% CI, 0.42 to 0.86; $P = .005$), respectively. Among the ER/PR-positive group, patients receiving trastuzumab had improved 3- and 5-year DFS of 7.2% (92.8; 95% CI, 90.1 to 95.7 *v* 85.6; 95% CI, 81.8 to 89.5) and 13.9% (89.1; 95% CI, 84.9 to 93.4 *v* 75.2; 95% CI, 69.3 to 81.5), respectively (Fig 4C). In the ER/PR-negative group, patients receiving trastuzumab also had improved 3- and 5-year DFS of 9.9% (83.0; 95% CI, 78.5 to 87.7 *v* 73.1; 95% CI, 67.7 to 78.8) and 12.0% (80.6; 95% CI, 75.5 to 86.0 *v* 68.6; 95% CI, 62.3 to 75.6), respectively (Fig 4D). There was no statistically significant interaction between hormone receptor status and study treatment ($P = .15$).

HRs were examined across the FISH *HER2*/CEP17 ratio subgroups in patients with ER/PR-positive and -negative tumors (Data Supplement). All subgroups had HRs of less than 1.0, but the patients with hormone receptor–positive tumors had lower HRs than patients with ER/PR-negative tumors, except for the *HER2* nonamplified patients with FISH ratios less than 2.0. Examining FISH ratio as a continuous covariate, the DFS benefit was similar between ER/PR-positive and -negative tumors and was not statistically significant.

DISCUSSION

This expanded analysis of our N9831 study results further confirms that patients with HER2-overexpressing and/or amplified tumors clearly benefit from adjuvant trastuzumab, administered concurrently with combination chemotherapy. Our analyses also elucidate important findings applicable to patient care. Among patients whose tumors had discordant central IHC and FISH results, those with HER2 protein overexpression and no gene amplification had an HR of 0.57 ($P = .56$; Fig 2B), and those with normal protein expression and gene amplification had an HR of 1.11 ($P = .86$; Fig 2B). Preanalytic challenges and the semiquantitative interpretation of IHC may obscure the importance of protein expression, as compared with the quantitative determination of gene copy number and gene/centromere copy number ratio by FISH. Our results are not statistically significant and warrant further follow-up.

We did not observe a linear dose-effect between the level of *HER2* gene amplification and trastuzumab response. Patients whose tumors had FISH ratios between 2 and less than 15 derived similar benefit from trastuzumab. These patients typically had tumors with normal chromosome 17 copy number (ratios 8 to 15) or with chromosome 17 polysomy (ratios 4 to 11). An HR of 0.96 was observed for patients whose tumors had FISH ratios more than 15, which typically had monosomy 17. A recent analysis of another adjuvant trastuzumab trial, Herceptin Adjuvant trial (HERA), also did not show a true linear dose response between level of *HER2* amplification and trastuzumab benefit.[31]

The relative benefit to adjuvant trastuzumab seems to be similar between patients whose tumors were found to be HER2 normal by IHC and FISH central testing (n = 103; HR, 0.51) and those patients whose tumors had HER2 protein overexpression (HR, 0.46) or gene amplification (HR, 0.49). However, we note that the DFS events are limited for the 103 patients, resulting in a nonsignificant HR ($P = .14$), and that these tumors had been described to be HER2 positive by the original local pathologist. This observation of a trend for benefit to

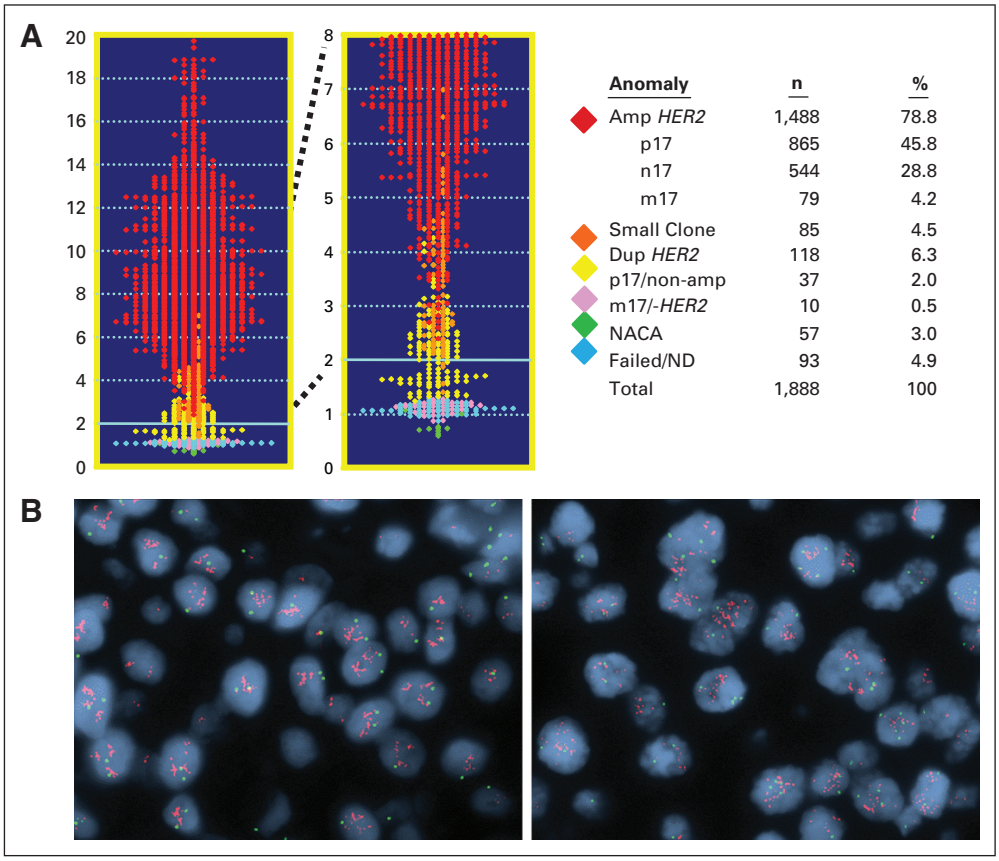

Fig 3. Distribution of human epidermal growth factor receptor 2 (*HER2*)/centromere enumerator probe for chromosome 17 (CEP17) ratios. (A) Distribution of *HER2*/CEP17 ratio (measured by fluorescent in situ hybridization [FISH]). Left, all patient data. Right, detailed findings for ratios between 0 and 8. (B) Illustration of amplification of *HER2* (red) and normal or polysomic CEP17 (p17; green) by FISH. Left, amplified *HER2* normal (disomic) chromosome 17 (n17). Right, amplified *HER2* p17. Amp, amplified; m17, monosomic chromosome 17; Dup, duplication; NACA, no apparent chromosome abnormality; ND, not determined.

trastuzumab independent of whether the tumors could be corroborated to be HER2 positive by a central laboratory may be explained by several factors: (1) limited number of patients and observed events; (2) enhanced chemosensitivity by combined chemotherapy and trastuzumab compared with trastuzumab alone in patients with low-to-intermediate tumor HER2 protein levels or gene copy number; (3) tumor HER2 heterogeneity, as 45% of specimens had different tissue blocks tested by the local and central laboratories; or (4) other mechanisms of action of trastuzumab, such as modulation of the immune system, possibly being more relevant in the adjuvant setting. The possibility of enhanced chemosensitivity is supported by preclinical data suggesting that cell lines with low-to-intermediate HER2 expression may have enhanced sensitivity to trastuzumab when combined with chemotherapy compared with trastuzumab alone.[32-35] Alternatively, anti-HER2 treatments may partially work via activity against the so-called BC stem cell, and not directly related to *HER2* gene amplification.[36,37] Of note is that our data are consistent with retrospective findings from the B-31 trial.[2,3] Bearing the caveats described above, if these data can be validated by larger studies, threshold values for HER2 positivity may then need to be redefined for patients receiving adjuvant chemotherapy with trastuzumab. Theoretically, the guidelines regarding adjuvant trastuzumab administration would then need to include many more new BC cases.

We found that p17 did not predict for trastuzumab benefit in patients with *HER2* amplification. Patients with *HER2*-amplified tumors, irrespective of p17 status, significantly benefitted from trastuzumab. This benefit was not significantly different between *HER2*-amplified/p17 and *HER2*-amplified/n17 patients ($P = .36$). Although, we cannot presently determine definitive associations between p17 and trastuzumab efficacy in patients with *HER2*-normal tumors (Data Supplement), we observed that *HER2*-normal patients with n17 seemed to benefit from trastuzumab ($P = .04$), whereas those with p17 did not appear to benefit from trastuzumab ($P = .85$). However, due to the limited number of *HER2*-normal patients/events, an interaction term cannot be defined. These results need to be interpreted with caution.

We did observe that in patients with *HER2* amplification and treated with chemotherapy alone, those with p17 benefited more than those with n17, suggesting that p17 may have some prognostic utility. Outside the stratification factors of hormone receptor and nodal status, the only significant covariate was tumor size and not Nottingham grade. Recent preliminary data from preclinical and independent non-HER2 directed clinical trials (MA5, BR9601, NEAT) support our findings that patients with p17 may have a better prognosis than those patients with n17 when treated with anthracyclines.[38,39]

Our data support previous findings that showed that patients with hormone receptor–positive and –negative BC have different patterns of relapse.[15] Although both cohorts in N9831 experienced a similar magnitude of benefit from trastuzumab therapy, irrespective of chromosome 17 status, the patients with hormone receptor–

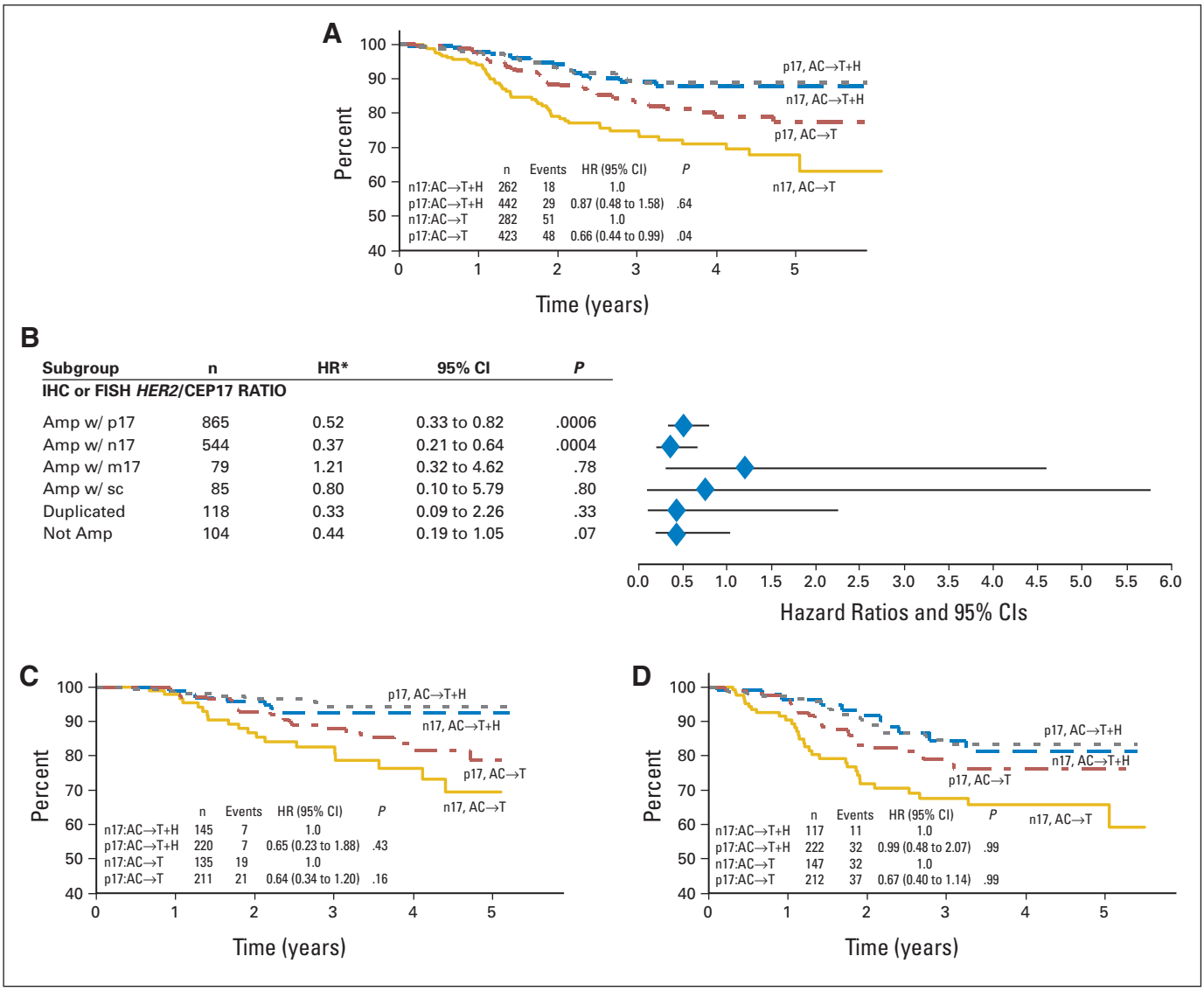

Fig 4. Survival analyses by chromosome 17 and hormone receptor status. (A) Kaplan-Meier curves for disease-free survival by arm and chromosome 17 status in patients with *HER2* amplification. The dashed gray line represents patients treated with trastuzumab (H; polysomic chromosome 17 [p17] background). The dashed blue line represents patients treated with H (normal [disomic] chromosome 17 [n17] background). The dashed red line represents patients treated without H (p17 background). The solid gold line represents patients treated without H (n17 background). (B) Disease-free survival hazard ratios (HR) by chromosome 17 status. The HRs were calculated comparing patients treated with and without H. Comparisons for each subgroup were performed using Cox proportional hazard models stratified by nodal status and hormone receptor status. (C) Kaplan-Meier curves for disease-free survival by arm and chromosome 17 status in patients with estrogen receptor (ER) –positive or progesterone receptor (PR) –positive tumors. The dashed gray line represents patients treated with H (p17 background). The dashed blue line represents patients treated with H (n17 background). The dashed red line represents patients treated without H (p17 background). The solid gold line represents patients treated without H (n17 background). (D) Kaplan-Meier curves for disease-free survival by arm and chromosome 17 status in patients with ER- and PR-negative tumors. The dashed gray line represents patients treated with H (p17 background). The dashed blue line represents patients treated with H (n17 background). The dashed red line represents patients treated without H (p17 background). The solid gold line represents patients treated without H (n17 background). AC, doxorubicin plus cyclophosphamide; T, paclitaxel; IHC, immunohistochemistry; FISH, fluorescent in situ hybridization; Amp, amplified; m17, monosomic chromosome 17; sc, small clone.

positive BC had a lower rate of relapse than patients with hormone receptor–negative BC (HR, 0.46; $P < .0001$) early in the follow-up period. However, these results should be interpreted with caution because longer follow-up is required. We recommend combining our data with data from other adjuvant trastuzumab-based clinical trials to analyze the various subpopulations.

In summary, our findings showed that accurate HER2 testing strategies are critical for appropriate management of patients with BC. We still advocate use of US Food and Drug Administration package insert guidelines for HER2 positivity. Newer ongoing studies may help determine whether new definitions of HER2 positivity or other biologic markers should be incorporated for decision to recommend trastuzumab in the adjuvant setting.[20,40]

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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