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ORIGINAL REPORT

Impact of PTEN Protein Expression on Benefit From Adjuvant Trastuzumab in Early-Stage Human Epidermal Growth Factor Receptor 2–Positive Breast Cancer in the North Central Cancer Treatment Group N9831 Trial

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See accompanying editorial on page 2073; listen to the podcast by Dr Rugo at www.jco.org/podcasts

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ABSTRACT

Purpose
It has been suggested that PTEN, a negative regulator of PI3K/AKT signaling, is involved in tumor sensitivity to trastuzumab. We investigated the association between tumor PTEN protein expression and disease-free survival (DFS) of patients randomly assigned to receive chemotherapy alone (arm A) or chemotherapy with sequential (arm B) or concurrent trastuzumab (arm C) in the phase III early-stage human epidermal growth factor receptor 2 (HER2) –positive trial—North Central Cancer Treatment Group (NCCTG) N9831.

Patients and Methods
The intensity and percentage of invasive cells with cytoplasmic PTEN staining were determined in tissue microarray sections containing three cores per block (n = 1,286) or in whole tissue sections (WS; n = 516) by using standard immunohistochemistry (138G6 monoclonal antibody). Tumors were considered positive for PTEN (PTEN-positive) if any core or WS had any invasive cells with ≥ 1+ staining. Median follow-up was 6.0 years.

Results
Of 1,802 patients included in this analysis (of 3,505 patients registered to N9831), 1,342 (74%) had PTEN-positive tumors. PTEN positivity was associated with hormone receptor negativity (χ^2 $P < .001$) and nodal positivity (χ^2 $P = .04$). PTEN did not have an impact on DFS within the various arms. Comparing DFS of arm C to arm A, patients with PTEN-positive and PTEN-negative tumors had hazard ratios (HRs) of 0.65 ($P = .003$) and 0.47 ($P = .005$), respectively (interaction $P = .16$). For arm B versus arm A, patients with PTEN-positive and PTEN-negative tumors had HRs of 0.70 ($P = .009$) and 0.85 ($P = .44$), respectively (interaction $P = .47$).

Conclusion
In contrast to selected preclinical and limited clinical studies suggesting a decrease in trastuzumab sensitivity in patients with PTEN-negative tumors, our data show benefit of adjuvant trastuzumab for patients with HER2-positive breast cancer, independent of tumor PTEN status.

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INTRODUCTION

Trastuzumab, a human epidermal growth factor receptor 2 (HER2) monoclonal antibody, has revolutionized the treatment of patients with HER2-positive breast cancer,[1] yet clinical resistance remains a significant problem.[2,3] Of the several markers hypothesized to predict sensitivity or resistance to trastuzumab, alteration of the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway, which can be activated by HER2, remains at the forefront of current research.[4-6]

The phosphatase and tensin homolog deleted from chromosome 10 (PTEN) tumor suppressor is a negative regulator of PI3K/AKT signaling, directly and indirectly affecting cell survival, proliferation, and apoptosis. PTEN dephosphorylates the 3′ end of the triphosphate PIP_3 in the inositol ring, resulting in the biphosphate PIP_2, which inhibits AKT activation and downstream signaling processes that depend on AKT for activation. Inactivation of PTEN, and thus lack of inhibition of the AKT-dependent processes, has been associated with tumorigenesis in multiple human cancers, including breast cancer.[6]

Loss of PTEN (defined differently by independent groups) has been observed in 22% to 64% of HER2-positive breast cancers.[4,7-11] Preclinical findings suggest that PTEN loss or inactivation confers resistance to trastuzumab.[7,9,12,13] However, data from retrospective analyses of small patient sets correlating PTEN alone or in combination with PI3K mutations have been conflicting.[4,7-11,14-17]

We investigated the incidence of PTEN protein expression and its correlation with patient clinicopathologic characteristics and trastuzumab sensitivity in the adjuvant breast cancer setting. Specifically, we evaluated the association between PTEN protein expression and disease-free survival (DFS) of patients with breast cancer randomly assigned to receive chemotherapy or chemotherapy with adjuvant trastuzumab in the phase III early-stage HER2-positive trial—North Central Cancer Treatment Group (NCCTG) N9831 (hereafter N9831).

PATIENTS AND METHODS

Patients

The N9831 trial had three arms: arm A, doxorubicin and cyclophosphamide followed by weekly paclitaxel; arm B, same as arm A followed by 1 year of sequential trastuzumab; and arm C, same as arm A with 1 year of concurrent trastuzumab, started the same day as weekly paclitaxel (Appendix Fig A1, online only). Women randomly assigned to the trastuzumab arm had a significantly increased DFS (stratified hazard ratio [HR], 0.52; 95% CI, 0.45 to 0.60; $P < .001$) and overall survival (OS; stratified HR, 0.61; 95% CI, 0.50 to 0.75; $P < .001$) compared with women assigned to the control arm.[1] In the N9831 comparison of sequential versus concurrent trastuzumab chemotherapy, there was an increase in DFS with concurrent trastuzumab (HR, 0.77; 95% CI, 0.53 to 1.11; $P = .02$). Although the number of events was lower than originally predicted when the trial was originally planned, the 5-year OS rate for the sequential arm was estimated at 89.7% (95% CI, 87.7% to 91.8%), and for the concurrent arm, it was estimated at 91.9% (95% CI, 90.0% to 93.7%).[1]

All tumors included in this report were tested for HER2 protein overexpression and gene amplification at a central laboratory (Mayo Clinic, Rochester, MN). Tumors were considered positive for HER2 according to US Food and Drug Administration–approved guidelines (immunohistochemistry [IHC]: circumferential strong 3+ membrane staining of > 10% invasive cells; fluorescent in situ hybridization: HER2:CEP17 ratio ≥ 2.0).[1,18-20] All patients signed informed consent forms. The Mayo Institutional Review Board and the Correlative Science Committee of the North American Breast Cancer Group (NABCG) approved this translational study.

Tissue Microarrays and Whole Tissue Sections

Tissue microarrays (TMAs) were constructed as part of the translational study component of N9831 by using an ATA-27 automated TMA construction system (Beecher Instruments, Silver Spring, MD), as described previously.[18] Each TMA (n = 1,286) contained biopsies from non-neoplastic human liver, placenta, and tonsil control tissues. Whole tissue sections (WSs; n = 516) were also examined from tumors not represented on TMAs, and a range of 0 to 3+ PTEN intensity staining was observed for both TMA sections and WSs.

PTEN Testing Methods

Standard laboratory protocols were followed for IHC. Antigen retrieval was performed on deparaffinized WS/TMA sections (5 μm) by using preheated citrate buffer (98°C; 40 minutes). Tissue sections were treated with Peroxidase Blocking Reagent (Dako, Carpenteria, CA) and Background Sniper (Biocare, Concord, CA) before manual IHC staining for PTEN (rabbit monoclonal; Cell Signaling, Boston, MA; 1:250; overnight incubation at room temperature[21]). Sections were transferred to a Dako Autostainer Plus (Dako Reference No. S3800) and incubated in secondary antibody (Dako Envision Plus Dual Link Horseradish Peroxidase Kit; Dako Reference No. K4061). The high-sensitivity diaminobenzidine (DAB+) Chromogenic Substrate System (Betazoid DAB, Biocare) was used for colorimetric visualization followed by counterstaining with hematoxylin.

PTEN positivity was defined as more than 0% of invasive cells with at least 1+ cytoplasmic staining. Because there is no validated standard definition for PTEN positivity or loss on the basis of our extensive literature review and personal discussions, we also examined an alternate cut point of more than 0% of invasive cells with at least 2+ cytoplasmic staining for PTEN positivity. This alternate cut point was considered because the cytoplasm of normal elements (when present) in the tissue (in more than 75% of patients)[22] typically stained at an intensity of 2+ (moderate), similar to previous reports.[23,24] The staining in normal elements and stroma was also used as a positive internal assay control, as applicable. The antibody used also produced slight nuclear staining that appeared to reflect in lesser degree the cytoplasmic expression, and was of unknown significance. The maximum cytoplasmic PTEN protein expression of the replicate TMA biopsies or the highest PTEN staining value across all parts of the WSs examined were used as the final result for all analyses associated with each patient outcome. Because WSs were used for patients not represented on TMAs, one result either from a TMA or a WS was used for each patient.

Statistical Methods

DFS (primary end point for N9831) was defined as local, regional, or distant recurrence, contralateral breast cancer, 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 as a result of any cause.[1] DFS duration was defined as the time from registration to the first DFS event. DFS was estimated by the Kaplan-Meier method. Comparisons among arms A, B, and C within subgroups were performed by using Cox proportional hazards models stratified by nodal status (1 to 3 *v* 4 to 9 *v* ≥ 10 positive nodes *v* positive sentinel node only *v* negative sentinel node with no axillary nodal dissection *v* axillary nodal dissection with no positive nodes) and hormone receptor status (estrogen receptor–positive and/or progesterone receptor–positive *v* negative for both). PTEN staining as a predictor of differential trastuzumab benefit among PTEN subgroups was tested by using Cox proportional hazards models, stratified by nodal status and hormone receptor status, including a treatment arm by PTEN subgroup interaction term.

RESULTS

Study Patients

The trial registered 3,505 patients onto arms A (1,232 patients), B (1,216 patients), and C (1,057 patients) of which 1,703 (A, 631; B, 566; C, 506) were excluded from this analysis for the following reasons: not HER2-positive by central pathology review (A,109; B, 90; C, 84); canceled before initiating therapy (A,15; B, 6; C, 7); did not meet eligibility criteria (A, 21; B, 23; C,17); no consent for future translational analysis (A, 65; B, 71; C, 55); withdrew consent/lost to follow-up (A, 65; B, 43; C, 21); no or inadequate tissue (A, 346; B, 319; C, 312); and technical failure (A, 10; B, 14; C, 10). Of the 3,505 patients, 1,802 (A, 601; B, 650; C, 551) were evaluable for PTEN protein expression (Appendix Fig A2, online only). The median follow-up time was 6.0 years and included all follow-up available through September 21, 2010. The clinicopathologic characteristics and outcomes of the 1,802 patients enrolled onto arms A, B, and C reported herein were similar to those of the 1,011 patients on arms A, B, and C excluded from analysis (Appendix Table A1, online only).

Clinicopathologic characteristics of the 1,802 patients whose tumors had 0 or 1 to 3+ PTEN cytoplasmic staining in any invasive tumor cell are listed in Table 1. Patients whose tumors were positive for PTEN cytoplasmic staining (ie, IHC 1 to 3+; PTEN positive) had a lower rate of hormone receptor positivity (45% *v* 55%; χ^2 $P < .001$) and a higher rate of nodal positivity (87% *v* 83%; χ^2 $P = .04$) than

Table 1. Patient Characteristics by Negative *v* Positive PTEN Cytoplasmic Staining

Characteristic	PTEN Cytoplasmic Staining (N = 1,802)				
	Negative (0) (n = 460; 26%)		Positive (1, 2, or 3+) (n = 1,342; 74%)		
	No.	%	No.	%	χ^2 *P*
Age, years					
Median	50		50		
Range	24-80		22-79		
Age group					.48*
< 40	84	18	234	17	
40-49	143	31	436	32	
50-59	137	30	445	33	
≥ 60	96	21	227	17	
Race/ethnicity					.06
White	382	83	1,163	87	
Other	78	17	179	13	
Menopausal status					.53
Premenopausal (or younger than age 50 years)	239	52	720	54	
Postmenopausal (or age 50 years or older)	221	48	622	46	
ER/PR status					< .001
ER positive or PR positive	253	55	603	45	
Other	207	45	739	55	
Surgery					.74
Breast conserving	176	38	525	39	
Mastectomy	284	62	817	61	
Nodal status					.28†
Node positive (1-3 positive nodes)	173	38	533	40	
Node positive (4-9 positive nodes)	107	23	354	26	
Node positive (≥ 10 positive nodes)	65	14	172	13	
Node negative (no positive nodes)	33	7	83	6	
Positive sentinel node	35	8	102	8	
Negative sentinel node	47	10	98	7	
Predominant tumor histology					.82‡
Ductal	433	94	1,271	95	
Lobular	14	3	39	3	
Other	9	3	31	2	
Missing	0	0	1	0.1	
Histologic tumor grade (Elston/SBR)					.69
Well/intermediate	133	29	375	28	
Poor	327	71	967	72	
Pathologic tumor size, cm					.69
< 2	150	33	424	32	
≥ 2	310	67	918	68	
Source of tissue					
Tissue microarray	241	52	1,045	78	
Whole section	219	48	297	22	

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; SBR, Scarff-Bloom-Richardson [breast cancer grading system].
*Mantel-Haenszel trend test.
†Negative *v* positive *P* = .04.
‡Missing not included in calculation.

those patients whose tumors had 0 PTEN cytoplasmic staining (ie, PTEN negative). Patients whose tumors had any 2 to 3+ PTEN cytoplasmic staining had a higher rate of hormone receptor positivity (60% *v* 48%; χ^2 $P < .001$) and higher rate of nodal positivity (90% *v* 83%; χ^2 $P < .001$) than those whose tumors had 0 to 1+ PTEN cytoplasmic staining (Appendix Table A2, online only). In addition, systematic differences may exist between the patients included on TMAs and patients analyzed by WSs (eg, tumor size) due, in part, to how patients were selected for TMAs. Specifically, tumor blocks were excluded from TMA construction if removal of the cores would have rendered the block unsuitable for additional translational analyses. Thus, statistical comparison of the rate of PTEN positivity between TMAs and WSs was not provided in Table 1.

Distribution and Heterogeneity of PTEN Protein Expression

Among 1,802 tumors, 26% (n = 460) had 0 PTEN cytoplasmic staining, 36% (n = 650) had 1+, 29% (n = 523) had 2+, and 9.4%

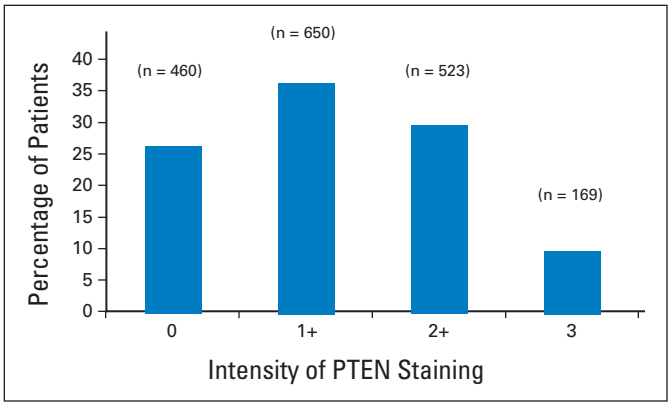

Fig 1. PTEN staining distribution for overall patient cohort.

(n = 169) had 3+ (Fig 1). Within the set of 1,181 patients represented by two or more scored cores on the TMA, agreement in the PTEN IHC scores (0 *v* 1+, 2+, 3+) across TMA cores was observed for 660 (56%) patients. Within the 516 patients represented by WSs, staining in 73 (14%) was considered heterogeneous, in which heterogeneity was defined as the percentage of staining at no single staining intensity (0, 1+, 2+, 3+) being greater than 50%. Of the 297 patients with WSs with PTEN staining (1+, 2+, 3+), heterogeneous staining was observed in 126 patients (42%). Nuclear and cytoplasmic 2 to 3+ staining were 71% concordant, and the Spearman correlation between cytoplasmic and nuclear staining intensity was 0.46 ($P < .001$; Appendix Table A3, online only). The relevance of nuclear staining is biologically unclear, and thus it was not analyzed for relationship with DFS. Representative staining patterns of PTEN protein expression are shown in Figure 2. An example of typical heterogeneity of PTEN expression in both normal breast epithelium and carcinomatous epithelium can be seen in Appendix Figures 3A to 3C (online only). Correlation between PTEN and HER2 protein expression is described in Appendix Table A4 (online only).

Associations Between PTEN Protein Expression and DFS

PTEN-negative (IHC 0) versus PTEN-positive (IHC 1 to 3+). No significant differences in DFS were observed between patients with PTEN-positive (IHC 1 to 3+) and PTEN-negative (IHC 0) tumors within any of the three arms by using the cut point of IHC 0 versus IHC 1 to 3+ (Table 2). In comparing DFS between arms C and A, patients with PTEN-positive and PTEN-negative tumors had HRs of 0.65 ($P = .003$) and 0.47 ($P = .005$), respectively (interaction $P = .16$; Figs 3A and 3B). In comparing DFS between arms B and A, patients with PTEN-positive and PTEN-negative tumors had HRs of 0.70 ($P = .009$) and 0.85 ($P = .44$), respectively (interaction $P = .47$; Figs 3A and 3B). In comparing DFS between arms C and B, patients with PTEN-positive and PTEN-negative tumors had HRs of 0.90 ($P = .49$)

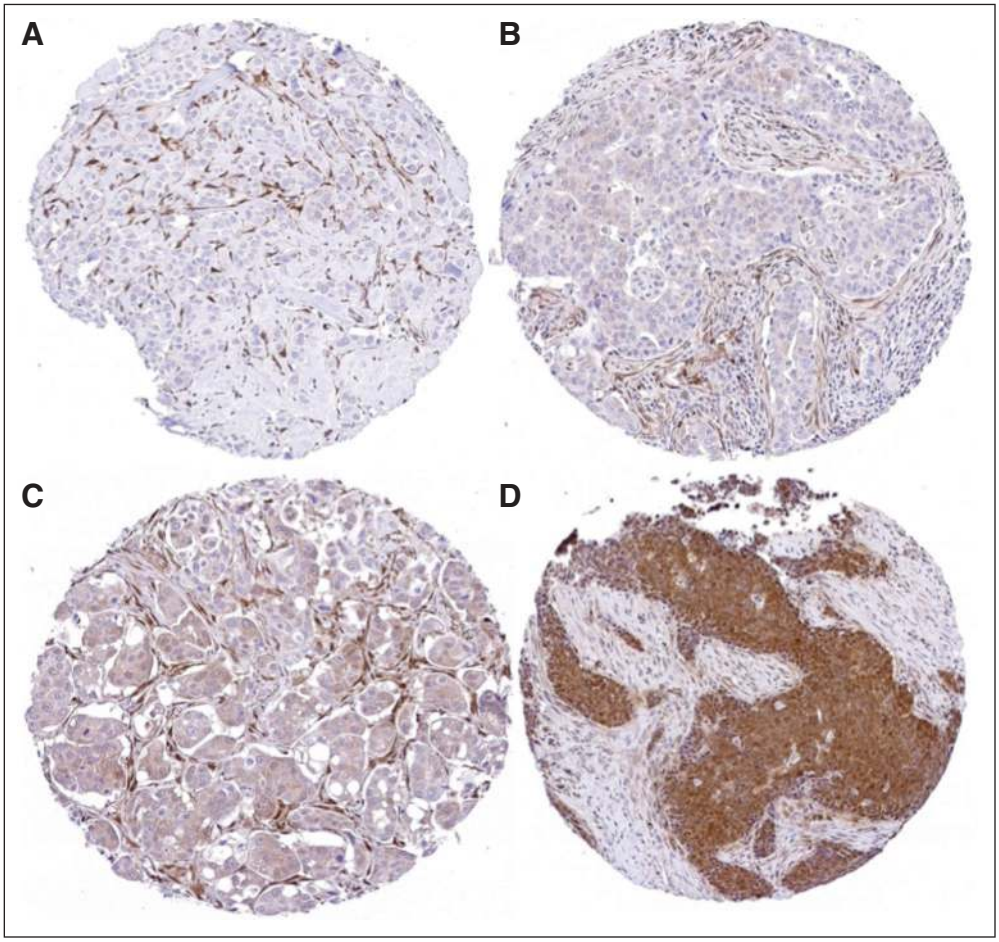

Fig 2. Representative PTEN staining. Immunohistochemical score of (A) 0, no cytoplasmic staining; (B) 1+, weak staining; (C) 2+, moderate staining; and (D) 3+, strong staining.

Table 2. DFS by PTEN Cytoplasmic Staining (stratified by hormone receptor and nodal status)

Arm	PTEN Cytoplasmic Staining Group	No. of Patients	No. of Events	HR	95% CI	*P*	DFS (months) 3-Year	DFS (months) 5-Year
PTEN neg (defined as IHC 0)	Neg (0)	460	110	1			85.4	78.4
	Pos (1, 2, or 3+)	1342	303	0.96	0.77 to 1.20	.70	85.4	80.0
A (n = 601)	Neg (0)	176	53	1			80.6	72.3
	Pos (1, 2, or 3+)	425	120	0.92	0.66 to 1.28	.64	81.4	73.9
B (n = 650)	Neg (0)	146	38	1			84.9	77.8
	Pos (1, 2, or 3+)	504	106	0.81	0.56 to 1.18	.27	86.3	81.0
C (n = 551)	Neg (0)	138	19	1			92.0	86.7
	Pos (1, 2, or 3+)	413	77	1.40	0.84 to 2.32	.20	88.3	85.1
PTEN neg (defined as IHC 0-1)	Neg (0 or 1+)	1110	259	1			84.9	78.8
	Pos (2 or 3+)	692	154	0.96	0.79 to 1.18	.70	86.1	80.9
A (n = 601)	Neg (0 or 1+)	361	109	1			79.2	71.5
	Pos (2 or 3+)	240	64	0.87	0.64 to 1.19	.38	84.1	76.4
B (n = 650)	Neg (0 or 1+)	401	95	1			84.5	78.6
	Pos (2 or 3+)	249	49	0.79	0.56 to 1.13	.19	88.3	83.0
C (n = 551)	Neg (0 or 1+)	348	55	1			91.4	86.6
	Pos (2 or 3+)	203	41	1.27	0.84 to 1.92	.25	85.7	83.7

Abbreviations: DFS, disease-free survival; HR, hazard ratio; IHC, immunohistochemistry; Neg, negative; Pos, positive.

and 0.56 ($P = .04$), respectively (interaction $P = .08$; Figs 3A and 3B). Similar associations were observed between PTEN status and DFS when examining only patients represented on TMAs (results not shown).

PTEN-negative (IHC 0, 1+) versus PTEN-positive (IHC 2+ to 3+). No significant differences in DFS were observed between patients with PTEN-positive and PTEN-negative tumors within any of the three arms by using the cut point of 0 to 1+ versus 2 to 3+ (Table 2). In comparing DFS between arms C (chemotherapy with concurrent trastuzumab) and A (chemotherapy alone), patients with PTEN-positive and PTEN-negative tumors had HRs of 0.70 ($P = .08$) and 0.50 ($P < .001$), respectively (interaction $P = .17$; Figs 3C and 3D). In comparing DFS between arms B and A, patients with PTEN-positive and PTEN-negative tumors had HRs of 0.68 ($P = .04$) and 0.78 ($P = .08$), respectively (interaction $P = .61$; Figs 3C and 3D). In comparing DFS between arms C and B, patients with PTEN-positive and PTEN-negative tumors had HRs of 1.01 ($P = .95$) and 0.66 ($P = .02$), respectively (interaction $P = .10$; Figs 3C and 3D). In addition, patients who had tumors with PTEN cytoplasmic staining of 0, 1+, 2+, and 3+ had HRs (arm C *v* A) of 0.47 (95% CI, 0.28 to 0.79), 0.53 (95% CI, 0.35 to 0.82), 0.71 (95% CI, 0.44 to 1.16), and 0.50 (95% CI, 0.23 to 1.11), respectively (Appendix Fig A4, online only).

DISCUSSION

PTEN is a tumor suppressor and a negative regulator of the PI3K/AKT survival pathway.[8,9,11,14,25] To the best of our knowledge, our report is the largest study (n = 1,802) that investigates the impact of PTEN expression on the benefit of trastuzumab and is the first to do so in the adjuvant setting.

In the N9831 tumor specimens, PTEN expression was heterogeneous and was characterized by both cytoplasmic and nuclear localization, similar to previous findings.[8,14] We observed that 26% of tumors analyzed from patients with HER2-positive breast cancer had no detectable protein expression of PTEN, and 74% had detectable cytoplasmic expression (defined as any 1+, 2+, or 3+ staining). If we define PTEN negativity as 0 to 1+ staining, then 62% of tumors had no or reduced expression, and 38% had PTEN protein expression similar to or higher than that observed in normal elements (defined as 2+). Our findings parallel data from previous studies by using IHC which showed that 22% to 64% of HER2-positive breast cancers express the PTEN protein to some degree, even in the setting of lack of standardized testing methodology in the literature.[4,7-10,26] By using our primary cut point of PTEN positivity defined as any staining (IHC score of 1+, 2+, or 3+), we observed that PTEN positivity was associated with a lower rate of hormone receptor positivity (45% *v* 55%) and a higher rate of nodal positivity (87% *v* 83%). In contrast, by using the ≥ 2+ cut point, PTEN cytoplasmic staining was associated with a higher rate of hormone receptor positivity (60% *v* 48%), analogous to previous reports in HER2-positive primary and metastatic breast cancer.[14,17,24,27]

In contrast to the hypothesis that PTEN loss is correlated with poor prognosis and decreased survival, our carefully conducted study demonstrated a lack of correlation of PTEN expression with outcome of N9831 patients. The DFS of patients within each treatment arm was not significantly different between patients with PTEN-positive and PTEN-negative tumors. We observed a benefit of concurrent trastuzumab compared with chemotherapy alone in all patients, independent of tumor PTEN protein expression. Our results in the adjuvant setting appear to conflict with a somewhat general consensus that PTEN loss correlates with trastuzumab resistance, although it is important to note that previously available correlative data have been inconsistent as well.

Nagata et al[8] reported that PTEN loss was associated with low rates of clinical response to trastuzumab treatment in 47 patients with metastatic breast cancer (no data on duration of response, progression-free survival [PFS], or OS). Fujita et al[9] also reported that loss of PTEN was associated with low rates of clinical response to trastuzumab plus paclitaxel treatment in 17 patients (no data on PFS or duration of response provided). Berns et al[7] reported data on 34 patients and

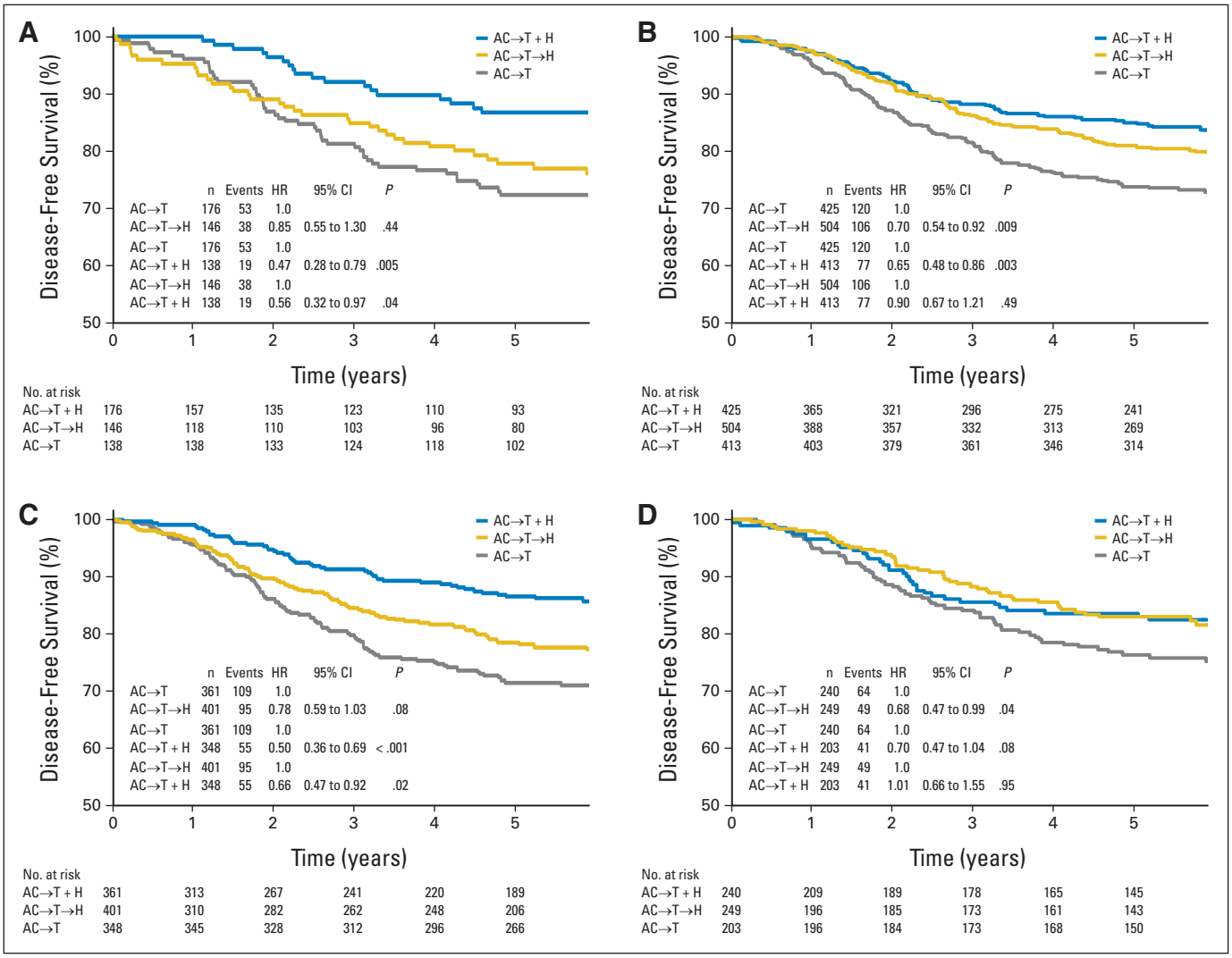

Fig 3. Kaplan-Meier curves of disease-free survival by treatment arm. (A) Negative (0), (B) positive (1, 2, or 3+), (C) negative (0 or 1+), and (D) positive (2 or 3+) PTEN cytoplasmic staining. AC, doxorubicin 60 mg/m^2 plus cyclophosphamide 600 mg/m^2 once every 3 weeks × 4; T, paclitaxel 80 mg/m^2/wk × 12 weeks; H, trastuzumab 4 mg/kg loading + 2 mg/kg/wk × 52 weeks; HR, hazard ratio.

found that those with either PI3K mutations or PTEN loss had shorter PFS than patients without those abnormalities; hazard ratios for each separate factor were not significant. Gori et al[10] evaluated PTEN and three other markers (EGFR, pMAPK, and pAKT) in 45 patients but did not find any significant correlations between these markers and clinical response to trastuzumab, time to progression, or OS. Fabi et al[4] studied PTEN, pAKT, and PI3K expression in 73 patients and reported a statistically nonsignificantly ($P = .06$) longer PFS for response to trastuzumab-containing therapy in patients with PTEN-positive tumors compared with patients with PTEN-negative tumors. Patients coexpressing PTEN and pAKT ($P = .01$) or coexpressing PTEN and PI3K ($P = .05$) had relatively significantly longer PFS compared with the remaining patients. Esteva et al[14] evaluated PTEN, pAKT, and PI3K mutations in 137 patients. They reported that none of the markers were independently predictive of response for the overall group of patients but that activation of the PI3K pathway (defined as PTEN loss and/or PIK3CA mutation) was significantly associated with poor response to trastuzumab and shorter survival time in patients receiving trastuzumab in the first-line metastatic setting. A recent study by Razis et al[25] had similar findings that demonstrated that PTEN loss and/or PIK3CA mutation was associated with decreased time to progression and survival in a group of 139 patients with metastatic disease with HER2-positive tumors treated with trastuzumab. Thus, an aggregate review of these published studies demonstrates inconsistent outcome correlations with PTEN protein alone or with PI3K mutations in the metastatic setting.[14]

Neoadjuvant studies also have yielded different conclusions.[11,15] The study by Yonemori et al[15] did not demonstrate a relationship between loss of PTEN expression or PTEN loss combined with pAKT expression and pathologic complete response in 44 patients who received trastuzumab-containing therapy. In contrast, the study of 31 patients by Dave et al[11] demonstrated that only four (18%) of the 22 patients with low PTEN expression or PIK3CA mutations and six (67%) of nine patients without low PTEN expression or PIK3CA mutations achieved pathologic complete response to trastuzumab plus docetaxel. Moreover, biomarker data from the neoadjuvant

HER2-positive NeoSphere study failed to find a correlation between PTEN protein (cytoplasmic or nuclear) or PI3K mutations with pathologic complete response to anti-HER2 therapy.[17] These neoadjuvant data are conflicting regarding the relationship of PTEN protein expression in response to trastuzumab-containing therapies.

Data regarding the role of PI3K mutation and outcome of patients from the FinHER phase III adjuvant study were recently reported. The investigators demonstrated a lack of association between PI3K mutations and relapse-free survival differences for patients who were randomly assigned to chemotherapy versus chemotherapy plus trastuzumab for early-stage HER2-positive breast cancer.[16]

In summary, literature studies have reported inconsistent correlative data of PTEN protein expression in tumor specimens from patients with HER2-positive breast cancer. Many studies used different antibodies and scoring methods, highlighting the need for standardized methodology and scoring criteria for reliable validation of biomarkers. In addition, the data in metastatic breast cancer studies have relied on tumor specimens obtained at the time of original diagnosis, not specimens from the metastatic tumors themselves. This latter point may be particularly significant because a recent investigation showed high discordance in PTEN levels (26%), *PIK3CA* mutations (18%), and hormone or HER2 status (25%) between matched primary tumor and metastases.[26]

Overall, the N9831 data indicate that PTEN protein expression alone (independent of cut point) is not significantly associated with prognosis or with differential benefit to concurrent trastuzumab. Importantly, the conflicting results obtained between this study in the adjuvant setting and some (not all) of the reported small studies in the metastatic and neoadjuvant settings highlight the need for accurate validation of biomarkers in large patient groups, with appropriate annotation for selecting treatment for patients and considering preanalytic variables, tissue sampling techniques, intratumoral heterogeneity, and cut point thresholds for biomarker negativity and/or positivity.[28] We are continuing protein expression and whole genome expression profiling studies of tumors from patients in N9831 to examine the association between DFS and a combination of markers to gain better understanding of the effect of altering signaling pathways on benefit from adjuvant trastuzumab and chemotherapy.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Appendix

Table A1. Patient Characteristics: Cohort *v* Noncohort

Characteristic	PTEN Cohort (n = 1,802; 64%) No.	%	Noncohort (no PTEN results) (n = 1,011; 36%) No.	%	χ^2 *P*
Age, years					
Median	50		49		
Range	22-80		23-82		
Age group					.67*
< 40	318	18	169	17	
40-49	579	32	342	34	
50-59	582	32	337	33	
≥ 60	323	18	163	16	
Race/ethnicity					.29
White	1,545	86	852	84	
Other	257	14	159	16	
Menopausal status					.55
Premenopausal (or younger than age 50 years)	959	53	550	54	
Postmenopausal (or age 50 years or older)	843	47	461	46	
ER/PR status					.15
ER positive or PR positive	856	48	452	45	
Other	946	53	559	55	
Surgery					.67
Breast conserving	701	39	385	38	
Mastectomy	1,101	61	626	62	
Nodal status					.45†
Node positive (1-3 positive nodes)	706	39	408	40	
Node positive (4-9 positive nodes)	461	26	268	27	
Node positive (≥ 10 positive nodes)	237	13	140	14	
Node negative (no positive nodes)	116	6	47	5	
Positive sentinel node	137	8	74	7	
Negative sentinel node	145	8	74	7	
Predominant tumor histology					.72‡
Ductal	1,704	95	958	95	
Lobular	53	3	31	3	
Other	44	2	20	2	
Missing	1	0.1	2	0.2	
Histologic tumor grade (Elston/SBR)					.96
Well/intermediate	508	28	286	28	
Poor	1,294	72	725	72	
Pathologic tumor size, cm					.11
< 2	574	32	352	35	
≥ 2	1,228	68	659	65	

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; SBR, Scarff-Bloom-Richardson [breast cancer grading system].
*Mantel-Haenszel trend test.
†Negative *v* positive $P = .06$.
‡Missing not included in calculation.

Table A2. Patient Characteristics by Negative *v* Positive PTEN Cytoplasmic Staining

Characteristic	PTEN Cytoplasmic Staining (N = 1,802)				
	Negative (0 or 1+) (n = 1,110; 62%)		Positive (2 or 3+) (n = 692; 38%)		
	No.	%	No.	%	χ^2 *P*
Age, years					
Median	50		50		
Range	22-80		23-79		
Age group					.71*
< 40	193	17	125	18	
40-49	361	33	218	32	
50-59	351	32	231	33	
≥ 60	205	18	118	17	
Race/ethnicity					.43
White	946	85	599	87	
Other	164	15	93	13	
Menopausal status					.22
Premenopausal (or younger than age 50 years)	578	52	381	55	
Postmenopausal (or age 50 years or older)	532	48	311	45	
ER/PR status					< .001
ER positive or PR positive	534	48	412	60	
Other	576	52	280	41	
Surgery					.71
Breast conserving	428	39	273	39	
Mastectomy	682	61	419	61	
Nodal status					.001†
Node positive (1-3 positive nodes)	430	39	276	40	
Node positive (4-9 positive nodes)	259	23	202	29	
Node positive (≥ 10 positive nodes)	150	14	87	13	
Node negative (no positive nodes)	83	7	33	5	
Positive sentinel node	81	7	56	8	
Negative sentinel node	107	10	38	5	
Predominant tumor histology					.08‡
Ductal	1,039	94	665	96	
Lobular	39	4	14	2	
Other	31	3	13	2	
Missing	1	0.1	0	0	
Histologic tumor grade (Elston/SBR)					.34
Well/intermediate	304	27	204	29	
Poor	806	73	488	71	
Pathologic tumor size, cm					.57
< 2	359	32	215	31	
≥ 2	751	68	477	69	
Source of tissue					
Tissue microarray	694	63	592	86	
Whole section	416	37	100	14	

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; SBR, Scarff-Bloom-Richardson [breast cancer grading system].
*Mantel-Haenszel trend test.
†Negative *v* positive $P < .001$.
‡Missing not included in calculation.

Table A3. Correlation Between Cytoplasmic and Nuclear PTEN Staining

	Cytoplasmic PTEN Staining (N = 1,802)						
	0		1+		2, 3+		
Nuclear PTEN Staining	No.	%	No.	%	No.	%	Total
0	440	36	483	39	309	25	1,232
1+	19	5	137	40	190	55	346
2, 3+	1	0.5	30	13	193	86	224
Total	460		650		692		1,802

NOTE. Spearman correlation 0.46 ($P < .001$).

Table A4. Correlation Between PTEN and HER2 Protein Expression

	PTEN Cytoplasmic Staining (N = 1,797)							
	0		1+		2, 3+		Total	
HER2 IHC	No.	%	No.	%	No.	%	No.	%
0	15	65	4	17	4	17	23	1
1+	8	30	11	41	8	30	27	2
2+	74	42	61	34	42	24	177	10
3+	358	23	574	37	638	41	1,570	87
Total	455	25	650	36	692	39	1,797	

NOTE. Spearman correlation 0.15 ($P < .001$).
Abbreviations: HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

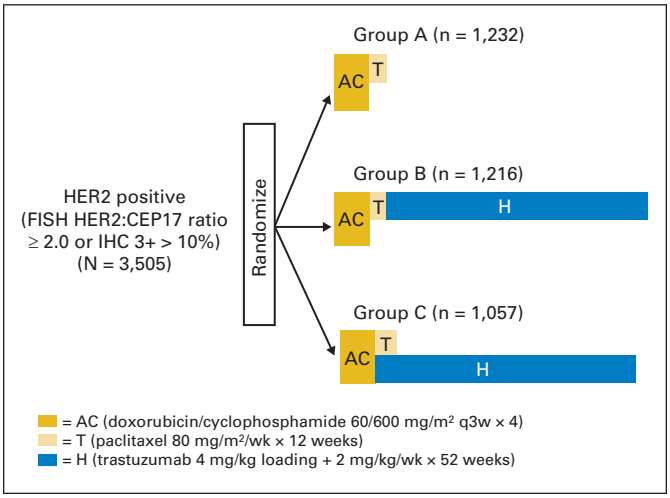

Fig A1. Schema for North Central Cancer Treatment Group (NCCTG) N9831 trial incorporating trastuzumab in adjuvant therapy. FISH, fluorescent in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; q3w, once every 3 weeks.

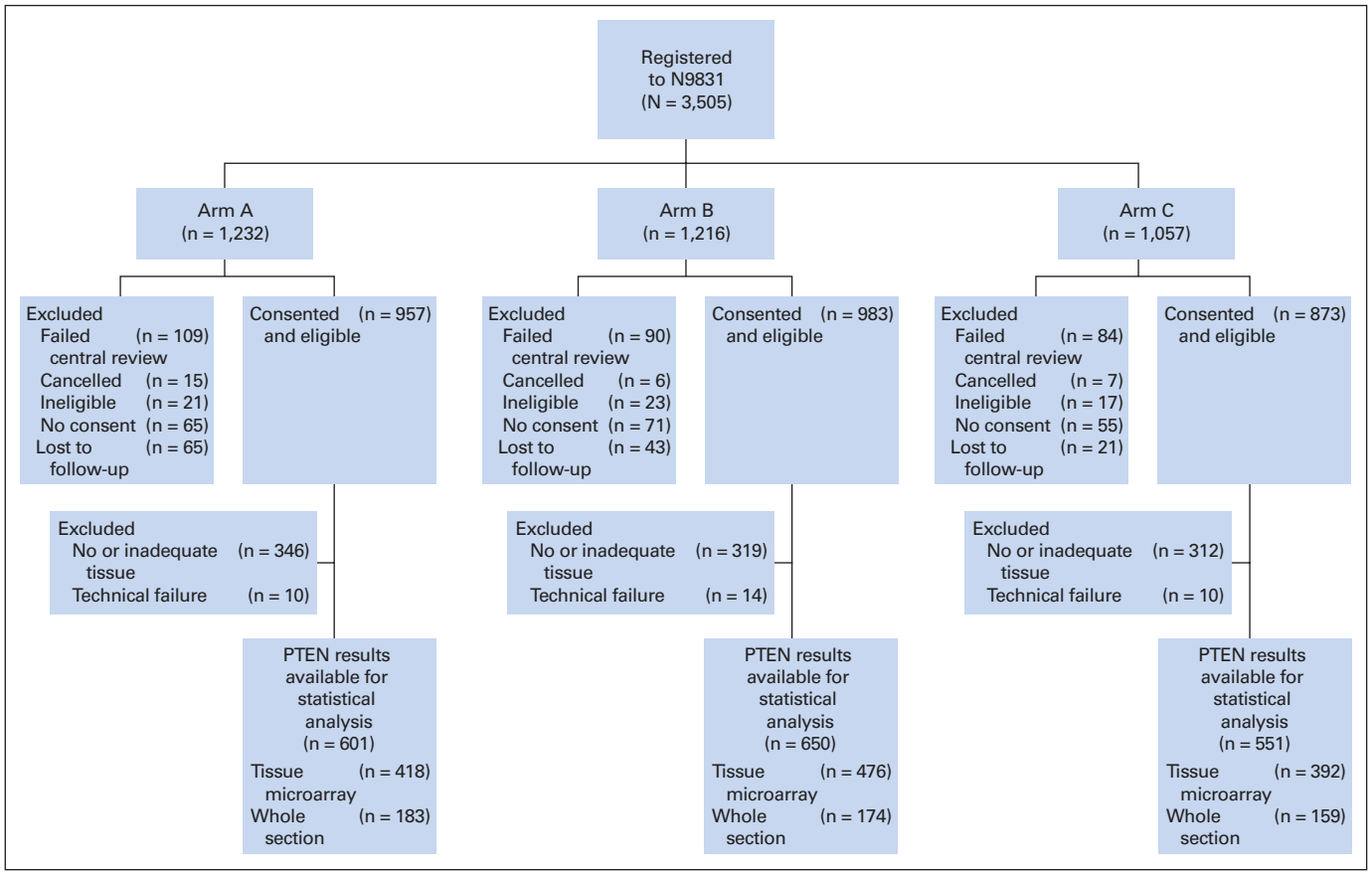

Fig A2. Patient flow diagram.

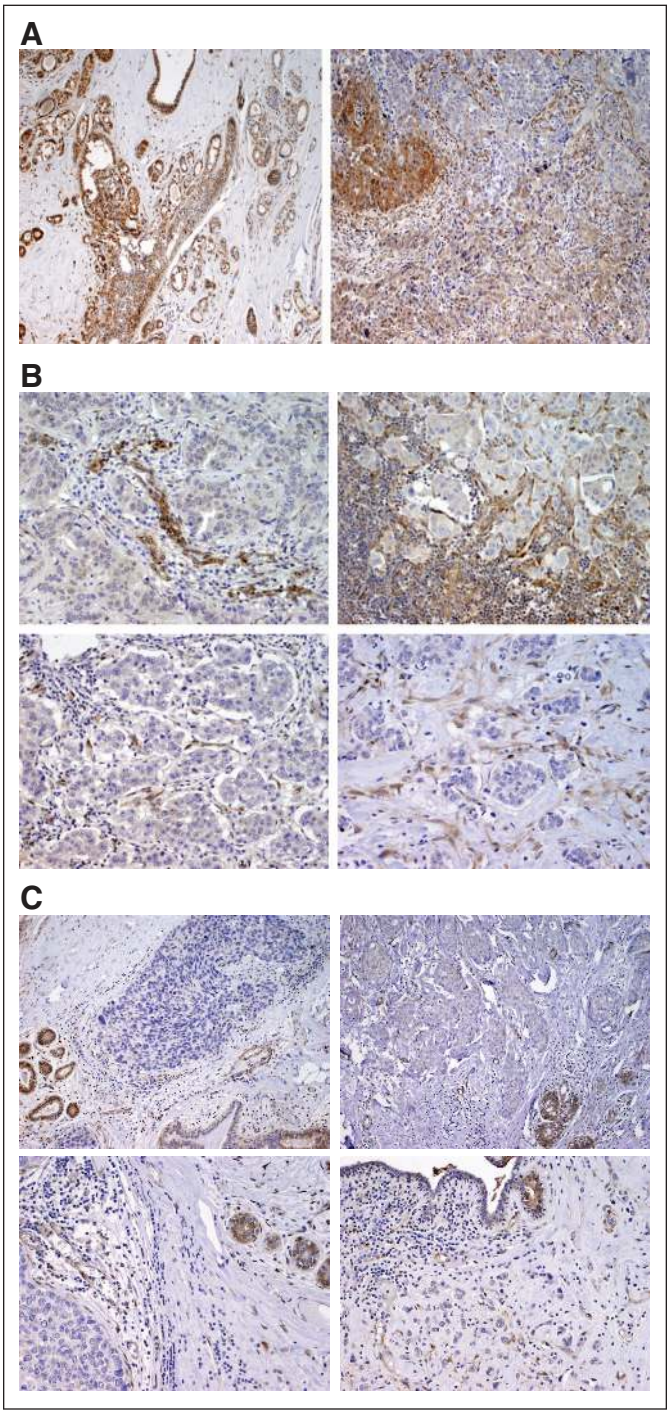

Fig A3. (A) PTEN variability in normal (left) and malignant (right) breast epithelium. Cell signaling anti-PTEN antibody 1:250, diaminobenzidine (DAB), ×100. (B) PTEN lost, positive endothelial internal control. Cell signaling anti-PTEN antibody 1:250, DAB, ×200. (C) PTEN lost, positive normal breast internal control. Cell signaling anti-PTEN antibody 1:250, DAB, ×200.

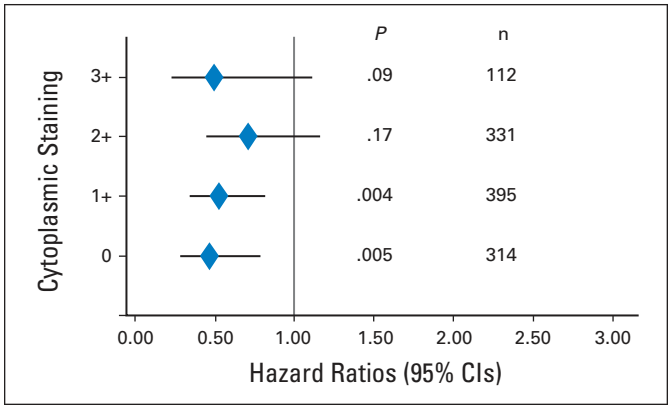

Fig A4. Forest plot: comparison of disease-free survival between concurrent trastuzumab and chemotherapy alone (arm C *v* arm A) within PTEN cytoplasmic staining subgroups.