original reports

NSABP B-47/NRG Oncology Phase III Randomized Trial Comparing Adjuvant Chemotherapy With or Without Trastuzumab in High-Risk Invasive Breast Cancer Negative for HER2 by FISH and With IHC 1+ or 2+

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abstract

PURPOSE Adjuvant trastuzumab reduces invasive breast cancer (IBC) recurrence and risk for death in patients with HER2-amplified or overexpressing IBC. A subset of patients in the landmark trastuzumab adjuvant trials who originally tested HER2-positive but were HER2-negative by central HER2 testing appeared to possibly benefit from trastuzumab. The objective for the NSABP B-47 trial was to determine whether the addition of trastuzumab to adjuvant chemotherapy (CRx) would improve invasive disease-free survival (IDFS) in patients with HER2-negative breast cancer.

PATIENTS AND METHODS A total of 3,270 women with high-risk primary IBC were randomly assigned to CRx with or without 1 year of trastuzumab. Eligibility criteria included immunohistochemistry (IHC) score 1+ or 2+ with fluorescence in situ hybridization ratio (FISH) < 2.0 or, if ratio was not performed, HER2 gene copy number < 4.0. CRx was either docetaxel plus cyclophosphamide or doxorubicin and cyclophosphamide followed by weekly paclitaxel for 12 weeks.

RESULTS At a median follow-up of 46 months, the addition of trastuzumab to CRx did not improve IDFS (5-year IDFS: 89.8% with CRx plus trastuzumab [CRxT] *v* 89.2% with CRx alone; hazard ratio [HR], 0.98; 95% CI, 0.76 to 1.25; $P = .85$). These findings did not differ by level of HER2 IHC expression, lymph node involvement, or hormone-receptor status. For distant recurrence-free interval, 5-year estimates were 92.7% with CRxT compared with 93.6% for CRx alone (HR, 1.10; 95% CI, 0.81 to 1.50; $P = .55$) and for overall survival (OS) were 94.8% with CRxT and 96.3% in CRx alone (HR, 1.33; 95% CI, 0.90 to 1.95; $P = .15$). There were no unexpected toxicities from the addition of trastuzumab to CRx.

CONCLUSION The addition of trastuzumab to CRx did not improve IDFS, distant recurrence-free interval, or OS in women with non-HER2–overexpressing IBC. Trastuzumab does not benefit women without IHC 3+ or FISH ratio-amplified breast cancer.

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ASSOCIATED CONTENT

Appendix

Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

Approximately 15% of invasive breast cancers are characterized by overexpression of the HER2 protein as a result of amplification of the *ERBB2* gene. Historically, HER2-positive disease has been associated with higher stage at presentation, greater relapse rates, and increased risk for breast cancer mortality without specific HER2-directed therapies.[1,2]

Trastuzumab, a humanized monoclonal antibody that targets the extracellular domain of the HER2 protein, induced response as a single agent and improved outcomes in combination with chemotherapy in the metastatic disease setting.[3,4] These responses occurred when immunohistochemistry (IHC) staining was 3+ intensity or fluorescence in situ hybridization (FISH) testing revealed a HER2 gene-to-centromere 17 ratio ≥ 2.0. Trastuzumab added to chemotherapy did not appear to improve outcomes in patients with metastatic breast cancer with HER2 IHC 2+ staining intensity and FISH ratios < 2.0.[5]

The landmark joint analysis of NSABP B-31 and NCCTG 9831 trials evaluating adjuvant trastuzumab

demonstrated major reductions in disease recurrence and death rates in patients with HER2 gene–amplified or protein-overexpressing breast cancer when 1 year of trastuzumab therapy was added to a standard adjuvant chemotherapy (AC) regimen of doxorubicin and cyclophosphamide followed by weekly paclitaxel (WP) for 12 weeks (AC→WP). The 10-year disease-free survival (DFS) rate was 73.7% in the trastuzumab-containing group compared with 62.2% in the nontrastuzumab group (hazard ratio [HR], 0.60; 95% CI, 0.53 to 0.68; $P < .001$), and the 10-year overall survival (OS) rate was 84% and 75.2%, respectively (HR, 0.63; 95% CI, 0.54 to 0.73; $P < .001$).[6,7] Similar findings were reported for the BCIRG 006[8] and HERA trials,[9,10] leading to a change in standard of care for adjuvant therapy in women with HER2-positive breast cancer.

Eligibility for the B-31 trial was based on HER2 testing results from local-site pathology laboratories. Central testing of archived tumor specimens from NSABP B-31 demonstrated that 174 of 1,787 cases (9.7%) had neither HER2 gene copy ratios ≥ 2.0 nor 3+ IHC overexpression of HER2. These patients appeared to derive similar magnitude of benefit from the addition of trastuzumab (relative risk for DFS, 0.34; 95% CI, 0.14 to 0.80; $P = .014$) compared with patients centrally confirmed to have HER2-positive tumors (P for interaction = .47). Data for OS trended toward significant interaction favoring the HER2-negative subset ($P = .08$). The relative risk comparing the trastuzumab-containing group with the nontrastuzumab group was 0.08 (95% CI, 0.01 to 0.64; $P = .017$) for patients with HER2 central assay–negative tumors, and 0.66 (95% CI, 0.43 to 0.99; $P = .047$) for patients with HER2-positive tumors.[11]

Similar findings were also reported from the N9831 trial, in which 103 cases were HER2 negative by both IHC and FISH by the central assay. With median follow-up of 5 years, these patients derived a similar degree of benefit as did the rest of the patients, with a similar HR for DFS (0.51) to that of patients with HER2-positive disease, although the trastuzumab effect did not reach statistical significance ($P = .14$).[12]

In the adjuvant setting, in which the immune system is less compromised, antibody-dependent cell-mediated cytotoxicity (ADCC) could be important in the activity of trastuzumab. The level of HER2 expression required for ADCC may be substantially lower than the currently defined threshold by clinical HER2 assays.[13,14]

In vitro, HER2 expression drives luminal breast cancer stem cells in absence of HER2 gene amplification. Although trastuzumab had no effects on the growth of established luminal breast cancer mouse xenografts, administration after tumor inoculation blocked subsequent tumor growth.[15]

These data suggested that the threshold of HER2 expression for benefit from adjuvant trastuzumab added to standard chemotherapy may be lower than that observed in the metastatic setting. NSABP B-47 was designed to determine whether the addition of trastuzumab to chemotherapy improves invasive disease-free survival (IDFS) in women with resected node-positive or high-risk node-negative breast cancer reported as HER2 1+ or 2+ and FISH negative (hereafter, HER2-low) on the basis of clinical HER2 testing performed at participating sites.[16]

PATIENTS AND METHODS

Study Design and Participants

NSABP B-47 was a phase III, multicenter, randomized adjuvant therapy trial. Eligible patients must have had resected node-positive or high-risk, node-negative, HER2-low invasive breast cancer. HER2-low was defined as having either an IHC score of 1+ (in situ hybridization testing not required) or 2+ with a negative HER2/chromosome 17 in situ hybridization ratio of < 2.0, or if ratio not performed, then a HER2 gene copy number of < 4.0. Patients must have had either a total mastectomy or lumpectomy with margins free of invasive tumor or ductal carcinoma in situ. For patients receiving the docetaxel plus cyclophosphamide (TC) regimen, the left ventricular ejection fraction requirement was ≥ 50% and for patients receiving the AC→WP regimen, the left ventricular ejection fraction requirement was ≥ 55%. Patients with previous history of breast cancer (except lobular carcinoma in situ), nonbreast malignancies (except for nonmelanoma skin cancers or in situ cancers treated only by local excision) within 5 years, or history of and/or active cardiac disease were not eligible. Patients with uncontrolled hypertension were not eligible and patients older than 50 years with controlled hypertension receiving antihypertensive medications could not receive AC→WP.

Investigators indicated upon entry which of the 2 chemotherapy regimens the patient would receive. The nonanthracycline regimen was TC (docetaxel 75 mg/m^2, cyclophosphamide 600 mg/m^2) administered intravenously (IV) every 3 weeks for 6 cycles. The anthracycline regimen was AC→WP (doxorubicin 60 mg/m^2 plus cyclophosphamide 600 mg/m^2 administered IV either every 3 weeks or every 2 weeks, per investigator discretion, for 4 cycles followed by WP 80 mg/m^2 IV administered weekly for 12 doses). Patients were randomly assigned using a biased-coin minimization algorithm to chemotherapy with or without trastuzumab therapy and were stratified by IHC score (1+ *v* 2+), pathologic nodal status (0 to 3, 4 to 9, ≥ 10), hormone-receptor status (estrogen receptor [ER]+ or progesterone receptor+ *v* both negative), and intended chemotherapy regimen (TC *v* AC→WP).

For patients who received TC chemotherapy, trastuzumab was given every 3 weeks during and after chemotherapy until 1 year after the first trastuzumab dose (8 mg/kg loading dose; 6 mg/kg for remaining doses). For patients who received AC→WP chemotherapy, trastuzumab began with the first dose of WP and was given weekly for 12 doses (4 mg/kg loading dose; 2 mg/kg for remaining weekly doses). After

completion of WP, trastuzumab continued with 6 mg/kg doses given every 3 weeks for a total of 1 year. Patients received adjuvant radiotherapy and endocrine therapy as clinically indicated.

Clinical assessments were required before each chemotherapy treatment cycle, every 9 weeks during post-chemotherapy trastuzumab therapy, at 6-month intervals for the first 5 years, and then annually through year 10. Annual mammograms were required when applicable. Adverse-event reporting was based on the descriptions and grading scales from the revised National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0, and was assessed at the end of each treatment cycle through 30 days after the last dose of chemotherapy or trastuzumab. Selected cardiac events and cardiac deaths were reported via the Cancer Therapy Evaluation Program Adverse Event Reporting System through 2 years after random assignment regardless of attribution and on a cardiac report form through year 10.

This study was approved by institutional review boards at participating clinical centers; written informed consent was provided.

Statistical Considerations

The primary end point for B-47 was IDFS, defined as the time from random assignment to local invasive recurrence after mastectomy, local invasive recurrence in the ipsilateral breast after lumpectomy, regional recurrence, distant recurrence, contralateral invasive breast cancer, second nonbreast primary cancer (excluding squamous or basal cell carcinoma of the skin), or death from any cause before recurrence or second primary cancer. Secondary end points included DFS, breast cancer–free survival (BCFS), recurrence-free interval (RFI), distant recurrence–free interval (DRFI), OS, and toxicity related to study regimens. The study also included components of menstrual history and inflammatory correlates, which will be reported separately.

B-47 was designed to have 90% power to detect a 33% reduction in the hazard rate of IDFS among the

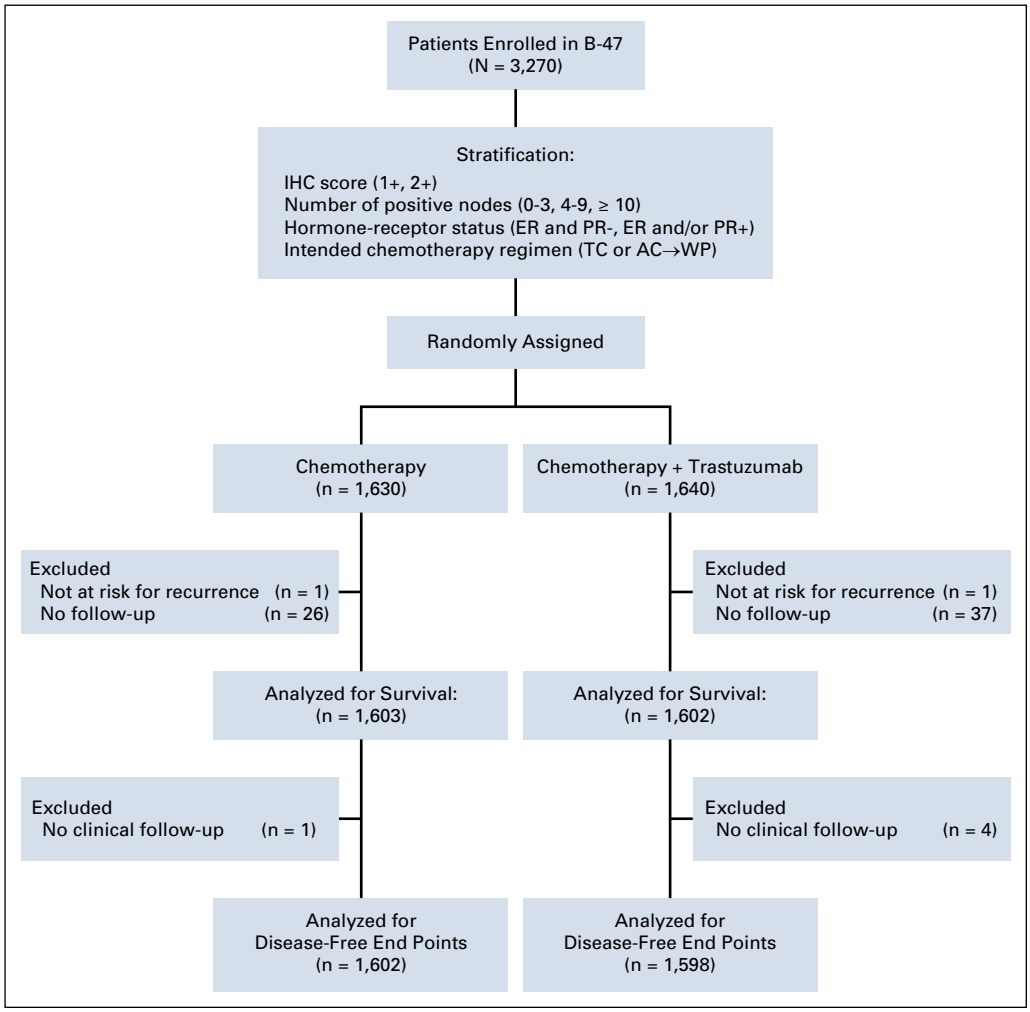

FIG 1. CONSORT diagram for the NSABP B-47 trial. AC→WP, doxorubicin and cyclophosphamide followed by weekly paclitaxel for 12 weeks; ER, estrogen receptor; IHC, immunohistochemistry; PR, progesterone receptor; TC, docetaxel plus cyclophosphamide.

TABLE 1. Patient and Tumor Characteristics for All Randomly Assigned Patients: NSABP B-47 Trial

	CRx		CRx + Trastuzumab		Total	
Characteristics	**No.**	**%**	**No.**	**%**	**No.**	**%**
Age at entry, years						
≤ 49	670	41.1	687	41.9	1,357	41.5
50-59	529	32.5	540	32.9	1,069	32.7
≥ 60	431	26.4	413	25.2	844	25.8
Race						
White	1,370	84.0	1,354	82.6	2,724	83.3
Black	144	8.8	175	10.7	319	9.8
Other/unknown	107	6.6	101	6.2	208	6.4
Multiracial	9	0.6	10	0.6	19	0.6
Ethnicity						
Not Hispanic or Latino	1,488	91.3	1,513	92.3	3,001	91.8
Hispanic or Latino	102	6.3	106	6.5	208	6.4
Unknown	40	2.5	21	1.3	61	1.9
IHC score						
1+	916	56.2	947	57.7	1,863	57.0
2+	714	43.8	693	42.3	1,407	43.0
Positive nodes, No.						
0	350	21.5	301	18.4	651	19.9
1-3	854	52.4	869	53.0	1,723	52.7
4-9	294	18.0	330	20.1	624	19.1
≥ 10	132	8.1	140	8.5	272	8.3
Hormone-receptor status						
ER and PgR negative	281	17.2	284	17.3	565	17.3
ER and/or PgR positive	1,349	82.8	1,356	82.7	2,705	82.7
Intended chemotherapy regimen						
AC→WP	909	55.8	916	55.9	1,825	55.8
TC	721	44.2	724	44.1	1,445	44.2
Histologic grade						
Low	129	7.9	165	10.1	294	9.0
Intermediate	705	43.3	703	42.9	1,408	43.1
High	792	48.6	770	47.0	1,562	47.8
Unknown	4	0.2	2	0.1	6	0.2
Type of surgery						
Lumpectomy	720	44.2	721	44.0	1,441	44.1
Mastectomy	836	51.3	859	52.4	1,695	51.8
Both	74	4.5	60	3.7	134	4.1
Total	1,630	100.0	1,640	100.0	3,270	100.0

Abbreviations: AC→WP, doxorubicin and cyclophosphamide followed by weekly paclitaxel for 12 weeks; CRx, adjuvant chemotherapy; ER, estrogen receptor; IHC, immunohistochemistry; PgR, progesterone receptor; TC, docetaxel plus cyclophosphamide.

chemotherapy-plus-trastuzumab group compared with the chemotherapy-alone group. Three preplanned interim analyses were performed when 66, 131, and 197 events were observed. To account for α spending and to preserve the overall type I error rate at 0.025, the adjusted 1-sided significance level for the final analysis was 0.0246. Therefore, a 2-sided P value $< .049$ for the primary end point was considered significant. All other P values were evaluated at

TABLE 2. Type of Invasive Disease–free Survival Events: NSABP B-47 Trial

Type of First IDFS Event	Treatment Group, No.		Total (N = 3,200)
	CRx (n = 1,602)	CRx + Trastuzumab (n = 1,598)	
Distant recurrence	69	73	142
Regional recurrence	10	7	17
Other local recurrence	8	5	13
Invasive IBTR	7	8	15
Invasive breast second primary	7	8	15
Nonbreast second primary	22	16	38
Death	10	11	21
Total IDFS events	133	128	261

Abbreviations: CRx, adjuvant chemotherapy; IBTR, ipsilateral breast tumor recurrence; IDFS, invasive disease–free survival.

the 2-sided .05 significance level. All analyses followed an intention-to-treat principle and included all at-risk women with available follow-up information. Distributions of time to IDFS as well as DFS, BCFS, RFI, DRFI, and OS for each treatment group were estimated by the Kaplan-Meier method and were compared between treatments by stratified log-rank tests (stratified by IHC score, number of positive nodes, hormone-receptor status, and intended chemotherapy regimen). HRs and 95% CIs were calculated from stratified Cox models. We performed interactions tests between treatment groups and each stratification variable. Analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC).

RESULTS

Patients

Between February 8, 2011, and February 10, 2015, a total of 3,270 patients were enrolled. Of these, 1,630 patients were randomly assigned to chemotherapy (CRx) and 1,640 to CRx plus trastuzumab (CRxT). This analysis used data collected through July 31, 2017. At that time, 3,205 at-risk patients had follow-up information available for OS, with 3,200 also having follow-up information for all other DFS end points (Fig 1). Median follow-up was 46 months. Baseline patient and tumor characteristics were well balanced between treatment groups (Table 1). In the CRx group, 84% of patients completed study therapy. In the CRxT group, 86% of patients completed planned CRx, 76% completed planned trastuzumab treatment, and 71% completed both.

Efficacy

As of July 31, 2017, a total of 261 IDFS events had been observed (CRx, n = 133; CRxT, n = 128). Breakdown by type of event is shown in Table 2. Addition of trastuzumab did not significantly improve IDFS compared with CRx (HR, 0.98; 95% CI, 0.76 to 1.25; $P = .85$; Fig 2A). Five-year point estimates for IDFS were 89.2% and 89.8% for the CRx and CRxT arms, respectively. The effect of adding trastuzumab to CRx on IDFS according to stratification factors is shown in Figure 3A. There was no evidence of a differential trastuzumab effect on IDFS by level of HER2 IHC expression, level of lymph node involvement, hormone-receptor status, or intended chemotherapy regimen.

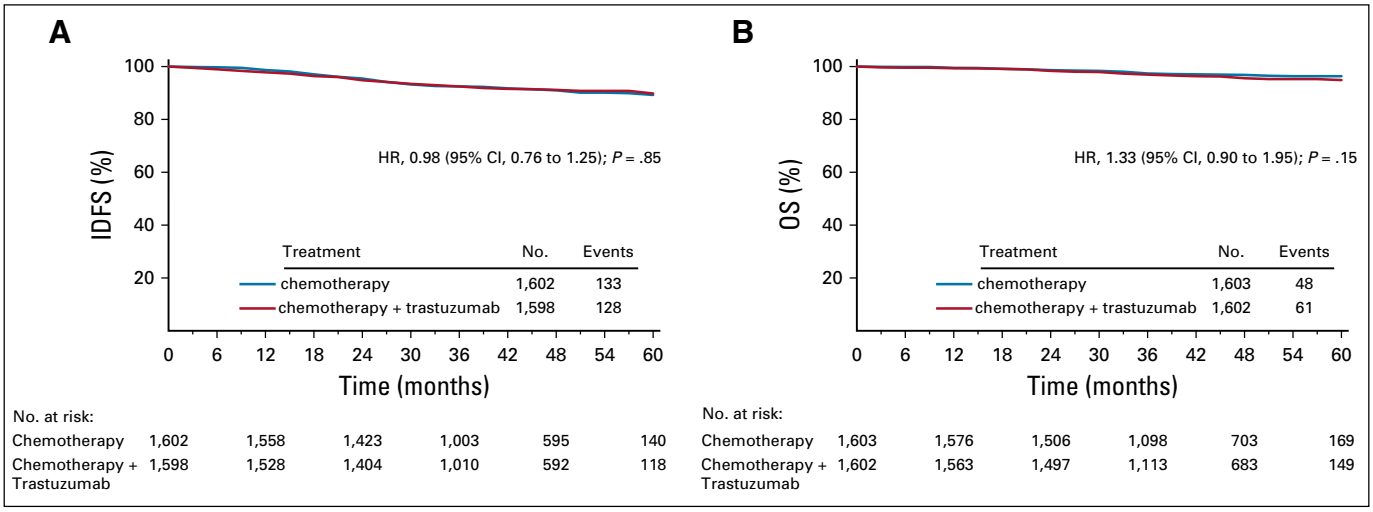

FIG 2. Results of Kaplan-Meier analyses for (A) invasive disease–free survival (IDFS) and (B) overall survival (OS) in the NSABP B-47 trial. HR, hazard ratio.

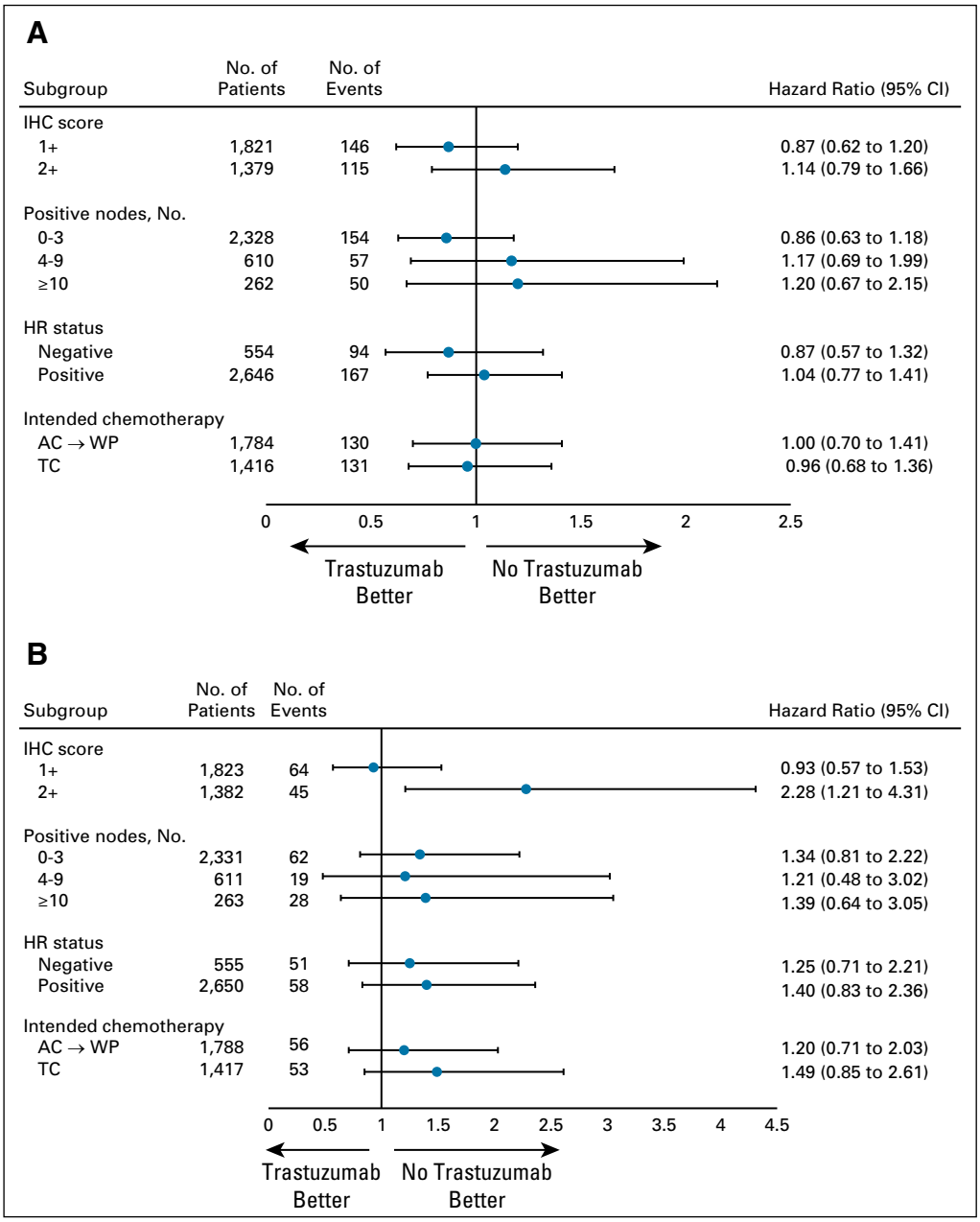

FIG 3. Hazard ratios for (A) invasive disease–free survival and (B) overall survival by stratification factors: NSABP B-47 trial. AC→WP, doxorubicin and cyclophosphamide followed by weekly paclitaxel for 12 weeks; HR, hormone receptor; IHC, immunohistochemistry; TC, docetaxel plus cyclophosphamide.

As of July 31, 2017, a total of 109 deaths had been observed (CRx, n = 48; CRxT, n = 61). There was no statistically significant difference in OS with the addition of trastuzumab to CRx (HR, 1.33; 95% CI, 0.90 to 1.95; $P = .15$; Fig 2B). Five-year point estimates for OS were 96.3% and 94.8% for the CRx and CRxT arms, respectively. The effect of adding trastuzumab to CRx on OS according to various subtypes is shown in Figure 3B. There was a significant interaction between HER2 IHC expression and treatment group ($P = .03$). There was no evidence of a differential trastuzumab effect on OS by level of lymph node involvement, hormone-receptor status, or chemotherapy regimen. Of the 109 total deaths, 78 were attributed to breast cancer (data not shown), with 32 in the CRx group and 46 in the CRxT group (HR, 1.51; 95% CI, 0.95 to 2.40; $P = .08$).

The addition of trastuzumab to CRx did not result in a statistically significant improvement in any of the secondary end points (data not shown). There were 267 DFS events observed, with 136 in the CRx group and 131 in the CRxT group (HR, 0.98; 95% CI, 0.77 to 1.24; $P = .84$). Five-year point estimates for DFS were 89.1% and 89.6% for the CRx and CRxT arms, respectively. There were 234 BCFS events observed, with 118 in the CRx group and 116 in the CRxT group (HR, 1.00; 95% CI, 0.77 to 1.30; $P = .99$). Five-year point estimates for BCFS were 91.0% and 90.7% for the CRx and CRxT arms, respectively. There were 194 RFI events observed, with 98 in the CRx group and 96 in the CRxT group (HR, 1.00; 95% CI, 0.75 to 1.33; $P = .98$). Five-year point estimates for RFI were 92.3% and 92.0% for the CRx and CRxT arms, respectively. There were 168 DRFI

TABLE 3. IHC Score, Invasive Disease-free Survival, and Overall Survival by Treatment Group and Hormone-Receptor Status: NSABP B-47 Trial

Outcome/Subgroup	No.	No. of Events (%)		Hazard Ratio*	95% CI	*P*
		IHC 1+	IHC 2+			
Invasive disease-free survival						
All patients	3,200	146 (8.0)	115 (8.3)	1.01	0.78 to 1.30	.96
Treatment group						
CRx	1,602	77 (8.6)	56 (8.0)	0.93	0.65 to 1.32	.67
CRx + trastuzumab	1,598	69 (7.5)	59 (8.7)	1.10	0.77 to 1.58	.61
Hormone-receptor status						
ER and PgR negative	554	58 (17.2)	36 (16.6)	0.97	0.63 to 1.50	.90
ER and/or PgR positive	2,646	88 (5.9)	79 (6.8)	1.03	0.75 to 1.40	.88
Overall survival						
All patients	3,205	64 (3.5)	45 (3.3)	0.96	0.65 to 1.42	.83
Treatment group						
CRx	1,603	33 (3.7)	15 (2.1)	0.60	0.33 to 1.12	.11
CRx + trastuzumab	1,602	31 (3.4)	30 (4.4)	1.37	0.81 to 2.31	.24
Hormone-receptor status						
ER and PgR negative	555	32 (9.5)	19 (8.8)	1.03	0.58 to 1.86	.91
ER and/or PgR positive	2,650	32 (2.2)	26 (2.2)	0.90	0.53 to 1.53	.71

Abbreviations: CRx, adjuvant chemotherapy; ER, estrogen receptor; IHC, immunohistochemistry; PgR, progesterone receptor.
*The hazard ratio compares the IHC 2+ group *v* IHC 1+ group.

events observed, with 81 in the CRx group and 87 in the CRxT group (HR, 1.10; 95% CI, 0.81 to 1.50; $P = .55$). Five-year point estimates for DRFI were 93.6% and 92.7% for the CRx and CRxT arms, respectively.

As an exploratory analysis, we examined the effect of having an IHC score of 2+ versus 1+ on IDFS and on OS. After comparing the distributions of patient characteristics between the 2 groups, we determined that the IHC 2+ group had more ER+ cancers, more patients treated with TC, and more high-grade tumors, compared with the IHC 1+ group (Table A1). In addition to nodal involvement, hormone receptor status, and intended chemotherapy, the histologic

TABLE 4. Adverse Events by Treatment Group: NSABP B-47 Trial

Adverse Event	CRx (n = 1,615)					CRx + Trastuzumab (n = 1,625)				
	Grade (%)					Grade (%)				
	0-1	2	3	4	5	0-1	2	3	4	5
Overall highest grade	9.1	53.5	33.1	4.1	0.2	5.9	46.6	42.5	4.6	0.4
Alopecia	60.9	39.1	0.0	0.0	0.0	59.9	40.1	0.0	0.0	0.0
Fatigue	61.6	34.3	4.1	0.0	0.0	60.8	35.5	3.7	0.0	0.0
Nausea	74.8	23.5	1.7	0.0	0.0	76.9	21.5	1.7	0.0	0.0
Peripheral sensory neuropathy	83.1	14.4	2.5	0.0	0.0	80.9	17.2	1.9	0.0	0.0
Diarrhea	86.3	11.0	2.7	0.0	0.0	80.1	15.8	4.1	0.1	0.0
Anemia	86.3	12.2	1.5	0.0	0.0	80.1	17.8	2.0	0.1	0.0
Mucositis oral	85.2	13.4	1.4	0.0	0.0	85.4	13.0	1.6	0.0	0.0
Bone pain	85.9	12.3	1.8	0.0	0.0	86.6	12.4	1.0	0.0	0.0
Myalgia	87.9	11.0	1.1	0.0	0.0	86.4	12.1	1.5	0.0	0.0
Arthralgia	90.3	8.9	0.9	0.0	0.0	85.8	13.1	1.1	0.0	0.0
Febrile neutropenia*	95.9	0.0	3.5	0.6	0.0	95.4	0.0	4.4	0.2	0.0

Abbreviation: CRx, adjuvant chemotherapy.
*Most common and of interest.

grade and treatment assignment were included as stratification variables for these comparisons. There were no statistically significant differences in IDFS or OS for IHC 2+ compared with IHC 1+ groups (Table 3).

Safety

Adverse event (AE) information was available for 3,240 randomly assigned patients (99%). This included 1,615 in the CRx group and 1,625 in in the CRxT group (Table 4). No new safety events for trastuzumab or CRx were seen, and both regimens were generally safe, as expected. Overall, 66 patients (4.1%) in the CRx group experienced a grade 4 AE as their highest reported grade, and 3 participants experienced a grade 5 AE (death). In the CRxT group, 75 patients (4.6%) experienced a grade 4 AE as their highest grade and 7 experienced a grade 5 AE. The grades 3 and 4 combined cardiac heart failure (CHF)/left ventricular systolic dysfunction AE rate was 2.3% with CRxT compared with 0.4% with CRx alone. In the CRxT arm, grades 3 and 4 CHF/left ventricular dysfunction was observed in 2.6% of patients treated with AC→WP and in 1.8% of patients treated with TC.

One patient who received trastuzumab and AC→WP died (grade 5 AE), which the treating physician attributed to CHF. The patient had been diagnosed with grade 3 CHF 2 months before; trastuzumab was discontinued, and symptoms improved. She continued to receive paclitaxel, was admitted with fevers and hypotension, and was treated with antibiotics and vasopressors. Upon central record review, it was determined that the patient died of sepsis.

DISCUSSION

The addition of trastuzumab to 2 standard CRx regimens for higher-risk primary breast cancer moderately expressing HER2 surface antigen in the absence of HER2 gene amplification did not significantly improve IDFS. These results did not differ by level of HER2 IHC expression (1+ or 2+), lymph node involvement, hormone-receptor status, or chemotherapy regimen.

This result from a large randomized trial refutes the outcome differences attributed to trastuzumab seen in 2 post hoc subset analyses of centrally tested HER2 nonamplified patients from prior large, trastuzumab adjuvant trials. Those patients, although not found to have HER2 amplification or HER-2 overexpression by the central laboratory, initially had local laboratory testing results that showed HER2 amplification by FISH or 3+ overexpression by IHC testing, which was a condition of clinical trial eligibility.

NSABP B-47 results were unequivocal for lack of benefit in both the IHC 1+ and 2+ cohorts, with no trend for benefit for the patients with IHC 2+ compared with those with IHC 1+ status. In fact, an exploratory analysis of OS by IHC status found OS of the patients with IHC 2+ status treated with trastuzumab to be statistically inferior to the IHC 2+cohort not treated with trastuzumab. This finding was unaffected by a sensitivity analysis for which histologic tumor grade was adjusted. Although the unexpected outcome of this exploratory analysis precludes definitive conclusions and we have no biologic reasoning to explain the findings, the results, coupled with the large 1,380-patient sample size of the IHC 2+ cohort and the greater number of IDFS events in that cohort, suggest it is highly unlikely that a subset of patients benefitting from trastuzumab is nested within the IHC 2+ cohort.

The initial finding of trastuzumab benefit in patients who were HER2-low on central testing in prior studies is not clearly explained by the B-47 results. Because the centrally tested tumor block could be a different block from the one used for original HER2 testing at the local sites, tumor heterogeneity could be one possible explanation. However, it is not likely, because among cases with available blocks from both primary tumor and lymph node metastasis, central assay results were identical. Furthermore, the rate of central HER2-assay negativity (ie, the false-positive rate if the central assay were regarded as the gold standard) improved from 18% to 6% after the protocol was amended to allow only qualified laboratories to provide HER2 testing for eligibility determination. The rate should have remained the same if the finding was due to submission of different blocks.

It is unlikely that the central testing results from B-31 were falsely negative. This is supported by the following observations: (1) central HER2-negative cases (defined as both IHC score $<$ 3+ and FISH ratio $<$ 2) expressed levels of HER2 mRNA that were identical to usual HER2-negative cases; (2) more importantly, microarray gene expression profiling demonstrated that these cases did not show HER2-enriched intrinsic molecular subtype with co-overexpression of genes such as GRB7, which are located next to the *ERBB2* gene and are usually coamplified and co-overexpressed in HER2-positive breast cancer; and (3) in an unpublished study, three independent pathologists reviewed central HER2-negative cases and agreed on 95% of the cases as HER2 negative.

Because trastuzumab binds to cell-surface protein, and reduced internal cell-signal transduction is difficult to demonstrate with trastuzumab, an immune mechanism of action in the adjuvant setting has been an appealing hypothesis. In vitro findings had suggested that the cell-surface HER2 antigen found in patients with IHC 2+ status could potentially support antibody-dependent cytotoxic cell death. The B-47 findings would support that low level of HER2 expression may not be enough to induce an immune reaction using trastuzumab. This study did not address whether a pure dimerization inhibitor molecule may have had activity in this setting.

The patient subset treated with docetaxel-containing chemotherapy plus trastuzumab had a lower level of left ventricular dysfunction than did the anthracycline subset. Lower rates of cardiotoxicity were observed in this study,

related to the more stringent eligibility criteria used. This confirms the relative cardiac safety of trastuzumab in early-stage breast cancer.

The B-47 results did not confirm the hypothesis that patients with HER2 IHC staining intensity of 1+ and 2+ in the absence of documented gene amplification could benefit from administration of trastuzumab with chemotherapy. Lack of benefit was evident in all patient subsets, and administration of HER2-directed therapies in adjuvant and neoadjuvant settings should remain limited to populations identified as HER2 positive according to ASCO/College of American Pathologists guidelines updated in 2018.[17]

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NSABP B-47/NRG Oncology Phase III Randomized Trial Comparing Adjuvant Chemotherapy With or Without Trastuzumab in High-Risk Invasive Breast Cancer Negative for HER2 by FISH and with IHC 1+ or 2+

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APPENDIX

TABLE A1. Patient and Tumor Characteristics for All Randomly Assigned Patients by IHC Score: NSABP B-47 Trial

	IHC 1+		IHC 2+		
Characteristic	**No.**	**%**	**No.**	**%**	***P***
Treatment group					.37
CRx	916	49.2	714	50.7	
CRx + Trastuzumab	947	50.8	693	49.3	
Age at entry, years					.13
≤ 49	800	42.9	557	39.6	
50-59	600	32.2	469	33.3	
≥ 60	463	24.9	381	27.1	
Race					.59
White	1,562	83.8	1,162	82.6	
Black	181	9.7	138	9.8	
Other/unknown	109	5.9	99	7.0	
Multiracial	11	0.6	8	0.6	
Ethnicity					.41
Not Hispanic or Latino	1,720	92.3	1,281	91.0	
Hispanic or Latino	110	5.9	98	7.0	
Unknown	33	1.8	28	2.0	
Positive nodes, No.					.96
0	366	19.6	285	20.3	
1-3	987	53.0	736	52.3	
4-9	357	19.2	267	19.0	
≥ 10	153	8.2	119	8.5	
Hormone-receptor status					.03
ER and PgR negative	345	18.5	220	15.6	
ER and/or PgR positive	1,518	81.5	1,187	84.4	
Intended chemotherapy regimen					.04
AC→WP	1,068	57.3	757	53.8	
TC	795	42.7	650	46.2	
Histologic grade					.002
Low	196	10.5	98	7.0	
Intermediate	792	42.5	616	43.8	
High	872	46.8	690	49.0	
Unknown	3	0.2	3	0.2	
Type of surgery					.39
Lumpectomy	830	44.6	611	43.4	
Mastectomy	964	51.7	731	52.0	
Both	69	3.7	65	4.6	
Total	1,863	100.0	1,407	100.0	

Abbreviations: AC→WP, doxorubicin and cyclophosphamide followed by weekly paclitaxel for 12 weeks; CRx, adjuvant chemotherapy; ER, estrogen receptor; IHC, immunohistochemistry; PgR, progesterone receptor; TC, docetaxel plus cyclophosphamide.