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Academia-Pharma Intersect: Breast Cancer

Neoadjuvant Doxorubicin/Cyclophosphamide Followed by Ixabepilone or Paclitaxel in Early Stage Breast Cancer and Evaluation of βIII-Tubulin Expression as a Predictive Marker

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Ixabepilone • Neoadjuvant • Biomarker • βIII-Tubulin • Early stage breast cancer

ABSTRACT

Background. This randomized phase II trial was designed to compare the rate of pathologic complete response (pCR) induced by neoadjuvant cyclophosphamide plus doxorubicin (AC) followed by ixabepilone or paclitaxel in women with early stage breast cancer (BC). Expression of βIII-tubulin as a predictive marker was also evaluated.

Patients and Methods. Women with untreated, histologically confirmed primary invasive breast adenocarcinoma received four cycles of AC followed by 1:1 randomization to either ixabepilone 40 mg/m^2 (3-hour infusion) every 3 weeks for four cycles (n = 148) or weekly paclitaxel 80 mg/m^2 (1-hour infusion) for 12 weeks (n = 147). All patients underwent a core needle biopsy of the primary cancer for molecular marker analysis prior to chemotherapy. βIII-Tubulin expression was assessed using immunohistochemistry.

Results. There was no significant difference in the rate of pCR in the ixabepilone treatment arm (24.3%; 90% confidence interval [CI], 18.6–30.8) and the paclitaxel treatment arm (25.2%; 90% CI, 19.4–31.7). βIII-Tubulin-positive patients obtained higher pCR rates compared with βIII-tubulin-negative patients in both treatment arms; however, βIII-tubulin expression was not significantly associated with a differential response to ixabepilone or paclitaxel. The safety profiles of both regimens were generally similar, although neutropenia occurred more frequently in the ixabepilone arm (grade 3/4: 41.3% vs. 8.4%). The most common nonhematologic toxicity was peripheral neuropathy.

Conclusions. Neoadjuvant treatment of early stage BC with AC followed by ixabepilone every 3 weeks or weekly paclitaxel was well tolerated with no significant difference in efficacy. Higher response rates were observed among βIII-tubulin-positive patients. ***The Oncologist*** 2013;18:787–794

Implications for Practice: Neoadjuvant chemotherapy is a common practice in early breast cancer treatment. One of the most important challenges in the clinic is to identify biomarkers to select patients for different therapies and achieve better outcomes. βIII-Tubulin expression is one of the well-established mechanisms of resistance to paclitaxel in vitro but limited clinical data exists regarding this issue. Single-agent neoadjuvant ixabepilone has previously demonstrated promising activity in invasive breast cancer, particularly in patients with high βIII-tubulin mRNA levels. In this phase II trial, neoadjuvant cyclophosphamide plus doxorubicin, followed by a direct comparison to either ixabepilone or paclitaxel (1:1) in women with early stage breast cancer, was well-tolerated with no significant difference in efficacy as measured by pathologic and clinical response. βIII-Tubulin expression measured by immunohistochemistry was not significantly associated with preferential benefit from ixabepilone versus paclitaxel treatment, suggesting that βIII-tubulin status defined by this technique should not be used for therapeutic decision-making in this patient population.

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Introduction

Neoadjuvant chemotherapy (NC) is a standard of care for locally advanced breast cancer (BC) and is being used increasingly in early stage disease [1, 2]. NC allows monitoring of responses and generates a unique scenario in which to search for biomarkers that could select patients for different therapies. It also increases the likelihood of breast-conservation surgery [3–5], without long-term impact on disease-free survival (DFS) or overall survival (OS) [6].

Pathologic complete response (pCR) is widely accepted as a prognostic indicator of favorable long-term outcome following NC [6, 7]. Patients with minimum residual disease after NC have outcomes similar to those of patients with a pCR [8]. Sequential administration of cyclophosphamide plus doxorubicin (AC), followed or preceded by a taxane, is used frequently and produces pCR rates of 20%–30% in unselected patient populations with early stage BC [1, 2]. Studies have shown improved pCR rates with sequential AC and docetaxel or paclitaxel (24% pCR) versus AC alone (14% pCR) or with sequential AC and docetaxel compared with a dose-dense concomitant schedule [1, 9–13].

Tumor sensitivity to taxanes varies, hence patient selection before therapy would be helpful. βIII-Tubulin expression is one of the well-established mechanisms of resistance to paclitaxel in vitro [14–18]. Limited clinical data also suggest that βIII-tubulin expression is a marker for paclitaxel resistance [14, 19–21].

Ixabepilone is a novel antitumor microtubule-stabilizing agent [22, 23] with low susceptibility to mechanisms that confer resistance to anthracyclines and taxanes, including high levels of βIII-tubulin, both in vitro and in xenograft models [24, 25]. In a phase II trial, single-agent neoadjuvant ixabepilone produced pCR rates of 18% (breast) and 11% (breast and auxiliary lymph nodes), with higher pCR observed in patients with estrogen receptor (ER)-negative tumors (29%) [26]. Further analysis of this trial showed higher pCR rates in patients with high βIII-tubulin mRNA levels [27]. These observations resulted in the hypothesis that paclitaxel-resistant cancers with high βIII-tubulin expression may be sensitive to ixabepilone.

This trial was designed to compare the rate of pCR induced by neoadjuvant AC followed by ixabepilone or paclitaxel in women with early stage BC. An additional primary objective was to compare pCR rates in different βIII-tubulin expression subsets as a predictive marker of treatment with ixabepilone relative to paclitaxel. Because pCR rates are generally higher in triple-negative BC (TNBC) and human epidermal growth factor receptor 2 (HER-2)-positive BC [28–31], these patient cohorts were also examined. In this paper, we present βIII-tubulin expression as assessed by immunohistochemistry (IHC); additional biomarker data are reported separately [32].

Materials and Methods

Patients

Women aged ≥18 years with untreated, histologically confirmed invasive breast adenocarcinoma stages T2–3, N0–3, and M0 (tumor size ≥2.0 cm) were eligible. Women with inflammatory BC, sensory/motor neuropathy, clinically significant cardiovascular disease, or serious intercurrent infection or nonmalignant medical illness were excluded. Initially, the trial was designed for patients with TNBC, but this criterion was later amended to include all tumor types.

Study Design and Treatment

This randomized, open-label, multicenter, phase II trial was conducted in accordance with the Declaration of Helsinki and in compliance with good clinical practice and local and national regulatory requirements (ClinicalTrials.gov identifier NCT00455533). This study was approved by the institutional review board or independent ethics committee at each site before enrollment; all patients provided written informed consent. Prior to chemotherapy, patients underwent a core needle biopsy of the primary cancer for molecular marker analysis. Subsequently, patients received four cycles of doxorubicin (60 mg/m^2 intravenously) and cyclophosphamide (600 mg/m^2 intravenously) every 3 weeks (Q3W) followed by 1:1 randomization to either ixabepilone (40 mg/m^2, 3-hour infusion) Q3W for four cycles or paclitaxel (80 mg/m^2, 1-hour infusion) weekly for 12 weeks. During randomization, patients were stratified by baseline tumor size (2–5 cm vs. >5 cm), ER status, clinical response to AC, and study site. The first dose of study drug was scheduled 21 days after the last dose of AC and after all AC-related toxicities resolved to baseline or grade 1. None of the HER-2-positive patients received NC with trastuzumab.

Doses of chemotherapy were reduced, temporarily withheld, or stopped depending on treatment toleration. Decreased doses of doxorubicin, cyclophosphamide, ixabepilone, or paclitaxel at the start of a cycle remained reduced for all subsequent cycles. There were no dose escalations or treatment crossovers during the study. Any patient who failed to recover from treatment-related toxicity to baseline or grade 1 (except grade 2 alopecia, myalgia, arthralgia, or fatigue) within 3 weeks of scheduled treatment was discontinued and advanced to the next stage. Colony-stimulating factors were used at the investigator's discretion but never as primary prophylaxis [33]. Patients progressing prior to or at completion of AC treatment were considered nonresponders for stratification purposes and were randomized to ixabepilone or paclitaxel. Patients discontinuing AC prior to the fourth cycle due to toxicity were evaluated for response and stratified and randomized to study treatment.

Patients underwent a lumpectomy and axillary lymph node dissection or modified radical mastectomy 4–6 weeks after the last dose of ixabepilone or paclitaxel. The surgical specimens were evaluated by a staff pathologist at each study site. No central pathology review was performed.

βIII-Tubulin IHC

βIII-Tubulin protein expression was measured by IHC using a prototype pharmacodiagnostic assay developed by Dako North America, Inc. (Carpinteria, CA). The assay was based on previously reported IHC assays for βIII-tubulin [34]. βIII-Tubulin cytoplasmic staining was scored on a 0–3 scale (negative, weak, moderate, strong), and the percentage of tumor cells at each intensity level was determined. Endothelial cells present in most tissue specimens were used as an internal positive control. An isotype-matched antibody was used as a negative control to evaluate background staining. A prespecified cutoff for βIII-tubulin-positive staining was defined as staining in

Table 1. Demographic and baseline characteristics (randomized patients)

Characteristic	Ixabepilone ($n = 148$)	Paclitaxel ($n = 147$)	Total ($N = 295$)
Age, yr, median (range)	48 (25–79)	46 (26–4)	48 (25–79)
Age <65 yr, *n* (%)	134 (90.5)	137 (93.2)	271 (91.9)
Age ≥65 yr, *n* (%)	14 (9.5)	10 (6.8)	24 (8.1)
Race, *n* (%)			
White	74 (50.0)	76 (51.7)	150 (50.8)
Black	7 (4.7)	4 (2.7)	11 (3.7)
Asian Indian	22 (14.9)	28 (19.0)	50 (16.9)
Chinese	16 (10.8)	12 (8.2)	28 (9.5)
Asian other	17 (11.5)	14 (9.5)	31 (10.5)
Other	12 (8.1)	13 (8.8)	25 (8.5)
Karnofsky performance status, *n* (%)			
100	107 (72.3)	103 (70.1)	210 (71.2)
90	28 (18.9)	39 (26.5)	67 (22.7)
80	13 (8.8)	5 (3.4)	18 (6.1)
Menopausal status, *n* (%)			
Premenopausal	71 (48.0)	75 (51.0)	146 (49.5)
Perimenopausal	6 (4.1)	6 (4.1)	12 (4.1)
Postmenopausal	67 (45.3)	64 (43.5)	131 (44.4)
Not reported	4 (2.7)	2 (1.4)	6 (2.0)
Tumor size classification, *n* (%)			
T1	1 (0.7)	2 (1.4)	3 (1.0)
T2	87 (58.8)	93 (63.3)	180 (61.0)
T3	60 (40.5)	52 (35.4)	112 (38.0)
Nodal classification			
N0	61 (41.2)	61 (41.5)	122 (41.4)
N1	68 (45.9)	68 (46.3)	136 (46.1)
N2	16 (10.8)	14 (9.5)	30 (10.2)
N3	3 (2.0)	4 (2.7)	7 (2.4)
ER status, *n* (%)			
Positive	60 (40.5)	58 (39.5)	118 (40.0)
Negative	88 (59.5)	88 (59.9)	176 (59.7)
Not reported	0	1 (0.7)	1 (0.3)
PR status, *n* (%)			
Positive	53 (35.8)	59 (40.1)	112 (38.0)
Negative	94 (63.5)	87 (59.2)	181 (61.4)
Not reported/unknown	1 (0.7)	1 (0.7)	2 (0.7)
HER-2 status, *n* (%)			
Positive	17 (11.5)	12 (8.2)	29 (9.8)
Negative	131 (88.5)	134 (91.2)	265 (89.8)
Not reported	0	1 (0.7)	1 (0.3)
TN status, *n* (%)	73 (49.3)	71 (48.3)	144 (48.8)

Abbreviations: ER, estrogen receptor; HER-2, human epidermal growth factor receptor 2; PR, partial response; TN, triple negative.

≥50% of tumor cells at an intensity of 2–3. In addition, the "histo-score" of βIII-tubulin staining was determined: 1 × (% cells with intensity 1) + 2 × (% cells with intensity 2) + 3 × (% cells with intensity 3).

Efficacy Assessment

The primary efficacy endpoint was pCR rate defined as the percentage of patients with no histologic evidence of residual invasive carcinoma in the breast and axillary lymph nodes, regardless of the presence or absence of ductal carcinoma in situ. Patients who received one dose or more of ixabepilone or paclitaxel and underwent surgery were evaluable for assessment. Secondary efficacy variables included objective response rate (ORR), breast-conservation rate, and residual cancer burden (RCB). Clinical response was assessed by a physician using breast calipers; for lesions not easily measurable, an alternative method was selected by the investigator (e.g.,

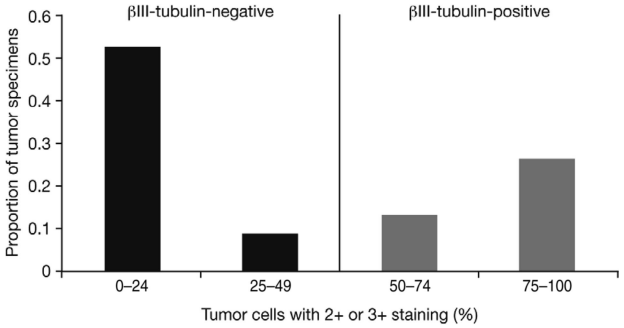

Figure 1. Bimodal distribution of percentage of βIII-tubulin tumor cell staining at ≥2+ across tumors.

mammography, ultrasound, computed tomography scan, and/or magnetic resonance imaging). ORR was defined as the proportion of patients with a clinical complete response (CR) or partial response (PR), graded according to the modified World Health Organization Tumor Response Criteria. Clinical response was assessed pretreatment, after AC treatment, and after ixabepilone or paclitaxel treatment prior to surgery. Patients receiving one dose or more of AC were evaluable for clinical response assessment to AC. The RCB was calculated and categorized, as described previously [8]. Category RCB-0 corresponds to pCR, and category RCB-1 corresponds to minimum residual cancer that carries the same favorable prognosis as pCR. These two categories were combined to form the secondary efficacy endpoint of RCB-0/RCB-1 rate.

Safety Assessment

Patients receiving one course of treatment or more were evaluated for safety. Adverse events (AEs) and laboratory tests were graded according to the National Cancer Institute Common Terminology Criteria of Adverse Events (version 3.0) and coded by system organ class or preferred term using the Medical Dictionary for Regulatory Activities (version 12.1). Toxicity with an onset after the first day of AC and prior to ixabepilone or paclitaxel was attributed to AC. Toxicity starting on the first day of ixabepilone or paclitaxel or up to 30 days after the last dose was attributed to the randomized study drug.

Statistical Analyses

The planned sample size for this study was 150 patients per treatment arm. The study had 81% power for detecting a ≥14% absolute difference in pCR rates between treatments (assuming a 23% pCR rate in the control arm), using a one-sided $\alpha = .05$ Fisher's exact test. Treatment arms were compared using a Cochran-Mantel-Haenszel (CMH) test stratified by tumor size, ER status, and response to AC. The pCR rate within each treatment arm and its exact 90% confidence interval (CI) was computed [35]. A logistic regression model with pCR as the response and βIII-tubulin expression, treatment, and interaction was used to evaluate any association between biomarker and treatment. The pCR rates were computed in the βIII-tubulin-positive and -negative subsets based on an optimal cutoff estimated using the cross-validation method, as were the pCR rates in the βIII-tubulin-positive and -negative subsets using a prespecified cutoff (defined as ≥50% 2+ or 3+ cells). βIII-Tubulin protein expression was summarized using descriptive statistics. Box plots with *p* values from a *t* test or one-way analysis of variance were generated to evaluate the association of βIII-tubulin expression with ER, PR, HER-2 status, TNBC, tumor classification, and nodal classification.

Results

Patients

From October 2007 to June 2009, 384 patients were enrolled at 59 centers in 15 countries. Of these, 313 patients received AC treatment, and 295 patients were subsequently randomized to ixabepilone or paclitaxel (supplemental online Fig. 1). Of the 295 patients, 289 were administered ixabepilone ($n = 145$) or paclitaxel ($n = 144$), and 12 patients did not undergo surgery. Demographic and baseline characteristics were generally well balanced between the treatment arms (Table 1). Four planned doses of AC were received by all but five patients, who received fewer cycles of AC because of disease

Table 2. Pathologic complete response by βIII-tubulin status (by immunohistochemistry) in evaluable patient populations

	Ixabepilone		Paclitaxel	
Patient group	***n***	**pCR rate (% [90% CI])**	***n***	**pCR rate (% [90% CI])**
All randomized	148	24.3 (18.6–30.8)	147	25.2 (19.4–31.7)
All treated	145	24.8 (19.0–31.4)	144	25.7 (19.8–32.4)
βIII-Tubulin-IHC status				
Positive[a]	43	34.9 (22.9–48.5)	42	35.7 (23.5–49.5)
Negative[a]	71	18.3 (11.2–27.5)	75	22.7 (15.0–32.0)
Positive[b]	—	35.9 (20.6–53.2)	—	36.1 (23.3–56.0)
Negative[b]	—	17.4 (10.3–27.1)	—	22.4 (13.1–32.0)
βIII-Tubulin-IHC status in ER-negative populations				
Positive[a]	32	43.8 (28.7–59.7)	34	41.2 (26.9–56.7)
Negative[a]	35	25.7 (14.1–40.6)	35	40.0 (26.0–55.3)

Abbreviations: —, not available; CI, confidence interval; ER, estrogen-receptor; IHC, immunohistochemistry; pCR, pathologic complete response.
[a]βIII-Tubulin positivity based on prespecified cutoff of staining in ≥50% of tumor cells at intensity of 2–3.
[b]Analysis based on cross-validation method using an optimal cutoff of staining in ≥46% of cells at intensity of 2–3. Because this was a resampling-based technique, the determination of individual sample size (*n*) is not applicable.

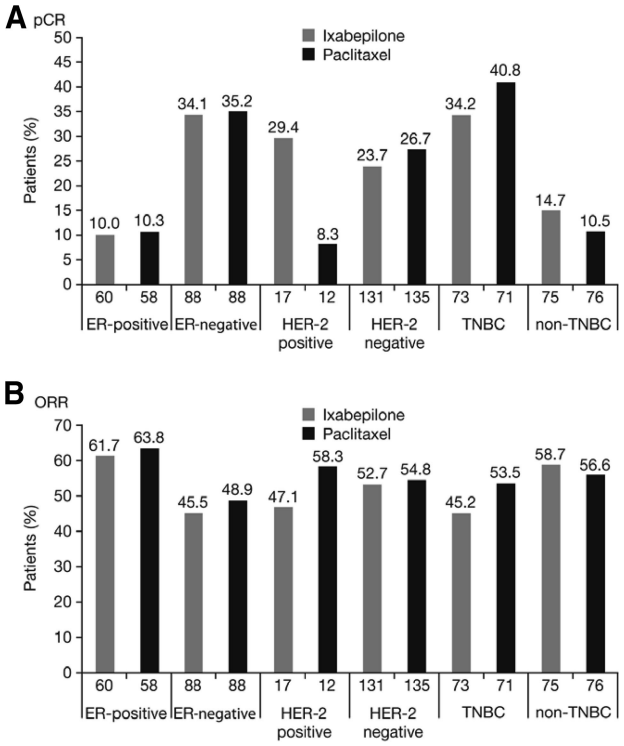

Figure 2. Comparison of ixabepilone and paclitaxel in pre-specified patient subsets (numbers below the bars are *n* values): pathologic complete response rate **(A)** and objective response rate **(B)** after treatment with study drug. HER-2 status was positive if the patient was either positive on fluorescence in situ hybridization or 3+ on immunohistochemistry; HER-2 negative refers to patients who did not meet these criteria.

Abbreviations: ER, estrogen-receptor; HER-2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ORR, objective response rate; pCR, pathologic complete response; PR, partial response; TNBC, triple-negative breast cancer (ER negative, PR negative, and HER-2 negative).

progression (PD; $n = 2$), toxicity ($n = 1$), or other reasons ($n = 2$). Seventy-two percent of patients were classified as responders to AC.

Most patients received ≥90% of their relative dose intensity of ixabepilone or paclitaxel (84.1% and 79.9% in the ixabepilone and paclitaxel arms, respectively). The median cumulative dose of ixabepilone was 159.2 mg/m^2 (range: 0.6–239.3 mg/m^2), and the median dose intensity per week was 13.3 mg/m^2 (range: 0.2–19.9 mg/m^2). The median cumulative dose of paclitaxel was 955.5 mg/m^2 (range: 84.5–1014.5 mg/m^2), and the median dose intensity per week was 78.4 mg/m^2 (range: 42.4–85.5 mg/m^2). Altogether, 124 patients (85.5%) received all 4 doses of ixabepilone, whereas 117 patients (81.3%) received all 12 doses of paclitaxel. The most common reasons for not receiving the planned doses of ixabepilone or paclitaxel were toxicity (5% in each arm) and PD (3.4% and 2.8% in the ixabepilone and paclitaxel arms, respectively). Eighteen patients (12.9%) in the ixabepilone arm and 18 patients (12.6%) in the paclitaxel arm had reduction of one dose or more (ixabepilone to 32 mg/m^2 and paclitaxel to 65 mg/m^2), mostly due to AEs (7.2% vs. 2.1%) and peripheral neuropathy (PN).

Table 3. Clinical objective response rate (randomized patients)

Response	Ixabepilone ($n = 148$)	Paclitaxel ($n = 147$)
Best response during treatment, *n* (%)		
CR	41 (27.7)	48 (32.7)
PR	79 (53.4)	66 (44.9)
Stable disease	14 (9.5)	17 (11.6)
Progressive disease	4 (2.7)	5 (3.4)
Unable to determine	10 (6.8)	11 (7.5)
ORR (90% CI)[a]	81.1 (75.0–86.2)	77.6 (71.2–83.1)
Tumor response at end of treatment,[b] *n* (%)		
CR	41 (27.7)	48 (32.7)
PR	36 (24.3)	33 (22.4)
Stable disease	41 (27.7)	40 (27.2)
Progressive disease	16 (10.8)	12 (8.2)
Unable to determine	14 (9.5)	14 (9.5)
ORR (90% CI)[a]	52.0 (45.0–59.0)	55.1 (48.0–62.1)

Abbreviations: CI, confidence interval; CR, complete response; ORR, objective response rate; PR, partial response.
[a]CI calculated using the Clopper and Pearson method.
[b]Response at the end of ixabepilone or paclitaxel treatment, with baseline tumor measurement at the end of treatment with neoadjuvant cyclophosphamide plus doxorubicin.

βIII-Tubulin Status (IHC)

Overall, 293 randomized patients had formalin-fixed paraffin-embedded tissue available for βIII-tubulin IHC assessment; however, in the submitted section, 43 patients had no evidence of tumor, and 3 patients had no tissue. Therefore, βIII-tubulin IHC data were available for 247 patients. Using a prespecified cutoff of ≥50% of cells with staining intensity of 2–3, the prevalence of βIII-tubulin-positive samples was 39.1% (Fig. 1), consistent with previous reports [14]. Distribution of the percentage tumor cells staining at ≥2+ intensity and the IHC histo-scores was bimodal (Fig. 1).

The association between βIII-tubulin status and BC subtype is detailed in supplemental online Table 1. In summary, 49% of patients (121 of 247) with βIII-tubulin IHC data were classified as triple negative (TN), 39% were ER positive, and 10% were HER-2 positive. Fifty-three percent of TN patients (64 of 121) were classified as βIII-tubulin positive compared with 22% of ER-positive patients (21 of 97) and 28% of HER-2-positive patients (7 of 25).

pCR Rates

There was no significant difference in pCR rate between the ixabepilone and paclitaxel arms at 24.3% (90% CI, 18.6–30.8) and 25.2% (90% CI, 19.4–31.7), respectively (CMH, $p = .8966$). Furthermore, there was no significant difference in pCR between treatments in subgroups defined by ER, HER-2, or TN status (Table 2; Fig. 2). Eighty-four percent (247 of 295) of patients had βIII-tubulin results available, and 231 of these had pCR information. The pCR rate and the cutoff for biomarker positivity were estimated using the cross-validation method. The optimal cutoff for βIII-tubulin positivity was estimated as ≥46% of tumor cells at 2+ or 3+ staining intensity. The percentage of βIII-tubulin-positive patients using this cutoff was

Table 4. Safety profile

	Ixabepilone ($n = 145$)			Paclitaxel ($n = 144$)		
Adverse Events	**All grades**	**Grade 3**	**Grade 4**	**All grades**	**Grade 3**	**Grade 4**
Deaths within 30 days of last dose	0	—	—	2 (1.4)	—	—
Any serious AE	17 (11.7)	8 (5.5)	6 (4.1)	11 (7.6)	6 (4.2)	3 (2.1)
Drug-related AE leading to discontinuation	14 (9.7)	7 (4.8)	4 (2.8)	13 (9.0)	4 (2.8)	1 (0.7)
Any treatment-related AE	130 (89.7)	46 (31.7)	15 (10.3)	121 (84.0)	24 (16.7)	3 (2.1)
Treatment-related nonhematologic AEs (≥10% incidence)						
Peripheral neuropathy[a]	63 (43.4)	6 (4.1)	0	72 (50.0)	5 (3.5)	0
Myalgia	41 (28.3)	4 (2.8)	0	19 (13.2)	1 (0.7)	0
Arthralgia	34 (23.4)	1 (0.7)	0	14 (9.7)	0	0
Bone pain	28 (19.3)	7 (4.8)	0	6 (4.2)	0	0
Fatigue	27 (18.6)	5 (3.4)	0	24 (16.7)	2 (1.4)	0
Diarrhea	25 (17.2)	2 (1.4)	0	18 (12.5)	2 (1.4)	0
Nausea	25 (17.2)	1 (0.7)	0	17 (11.8)	0	0
Musculoskeletal pain	15 (10.3)	1 (0.7)	0	6 (4.2)	1 (0.7)	0
Hematologic laboratory toxicities						
Neutropenia[b]	110 (76.9)	36 (25.2)	23 (16.1)	77 (53.8)	12 (8.4)	0
Leukopenia	111 (77.6)	44 (30.8)	8 (5.6)	111 (77.6)	7 (4.9)	0
Thrombocytopenia	34 (23.8)	1 (0.7)	0	9 (6.3)	1 (0.7)	0
Anemia	130 (90.9)	2 (1.4)	0	135 (94.4)	6 (4.2)	1 (0.7)

Abbreviation: —, not available; AE, adverse event.
[a]Peripheral neuropathy was defined as a composite of neuropathy AEs based on the Medical Dictionary for Regulatory Activities (version 12.1).
[b]Febrile neutropenia was uncommon in both study arms (one patient in each study arm).

determined to be 39.4%. The pCR rate in the βIII-tubulin-positive subgroup was 35.9% (90% CI, 20.6–53.2) with ixabepilone and 36.1% (90% CI, 23.3–56.0) with paclitaxel. These observations are consistent with the original phase II ixabepilone neoadjuvant data, in which pCR rates were significantly higher in the βIII-tubulin-positive cases [27]. βIII-Tubulin expression, however, had no ability to distinguish between ixabepilone versus paclitaxel sensitivity (interaction p value between βIII-tubulin expression and treatment, $p = .5757$). In addition, results were consistent in the ER subset and did not show any detectable interaction between βIII-tubulin expression and paclitaxel or ixabepilone treatment ($p = .5112$).

A plot of interaction p values against the prevalence rate of βIII-tubulin IHC positivity defined by tumor cell percentage staining at ≥2+ was created (supplemental online Fig. 2). Regardless of IHC cutoff, the interaction between βIII-tubulin staining and treatment effect was not significant. The cutoff with the lowest interaction p value ($p = .34$) was 40% of tumor cells with ≥2+ staining intensity.

RCB and Clinical ORR

The combined RCB-0/RCB-1 rate was not significantly different between the ixabepilone and paclitaxel arms at 30.4% (90% CI, 24.2–37.2) and 33.3% (90% CI, 26.9–40.3), respectively (CMH, $p = .5806$). Furthermore, the clinical ORR was similar. In the ixabepilone arm, the ORR was 81.1% (90% CI, 75.0–86.2) including 41 patients (27.7%) with CR and 79 (53.4%) with PR. In the paclitaxel arm, the ORR was 77.6% (90% CI, 71.2–83.1) including 48 patients (32.7%) with CR and 66 (44.9%) with PR (Table 3). There was no difference in response rate by treatment within any of the prespecified patient subsets (Fig. 2). The rate of breast-conservation surgery was 41.9% (90% CI, 35.1–49.0) with ixabepilone and 32.7% (90% CI, 26.3–39.6) with paclitaxel.

Safety

The safety profile of the ixabepilone arm was similar to that of the paclitaxel arm, except that grade 3/4 neutropenia occurred more frequently with ixabepilone (41.3% vs. 8.4%; Table 4). Febrile neutropenia occurred in one patient (0.7%) in each treatment arm. Grade 3/4 anemia and thrombocytopenia occurred infrequently. The commonest nonhematologic toxicity was PN, occurring in 43.4% and 50.0% (treatment related, all grades) of patients who received ixabepilone and paclitaxel, respectively. Eleven patients had grade 3 events, six patients in the ixabepilone arm and five patients in the paclitaxel arm. No grade 4 PN was reported. Three deaths occurred during the study in the paclitaxel arm, including two deaths within 30 days of the last dose. One patient died due to drug-related sepsis, acute respiratory distress syndrome, disseminated intravascular coagulation, and cardiorespiratory arrest; the other patient died due to aspiration of gastric content. The third patient died due to PD with brain metastases.

Serious AEs, regardless of relationship to study drug, were reported in 17 patients (11.7%) in the ixabepilone arm and 11 patients (7.6%) in the paclitaxel arm. Fourteen patients (9.7%) discontinued ixabepilone due to drug-related AEs, the most common peripheral motor neuropathy (PMN; 2.1%), peripheral sensory neuropathy (PSN; 2.1%), and hypersensitivity (1.4%). In comparison, 13 patients (9.0%) discontinued paclitaxel due to drug-related AEs, frequently due to PMN (2.8%), increased alanine aminotransferase (2.8%), and PSN (2.1%).

Discussion

This is the first randomized neoadjuvant trial to compare the efficacy of ixabepilone versus paclitaxel following AC in early stage BC. The primary efficacy measure of pCR, as well as the secondary efficacy measures of clinical response, RCB, and breast-conservation rate, were similar between the two treatment arms. Both drugs were similarly effective in ER-negative and ER-positive patents. However, both drugs had higher rates of pCR in ER-negative cancers compared with ER-positive cancers. Previously reported trials of NC with four courses of single-agent ixabepilone produced a pCR rate of 11% [26], which is similar to rates with single-agent paclitaxel in this setting [36]. Use of ixabepilone (or paclitaxel) after four courses of AC increased the pCR rate considerably. This is consistent with previous reports that showed longer, sequential taxane-containing regimens had higher pCR rates than AC [1, 9, 12, 13].

The study showed that βIII-tubulin was a marker for chemosensitivity and was not associated with preferential benefit from ixabepilone versus paclitaxel treatment. Results of preclinical models and retrospective clinical studies indicated that increased expression of βIII-tubulin may be a marker of taxane resistance [14–16, 19, 20, 25]. Furthermore, some xenograft studies suggested that ixabepilone was more active than paclitaxel in the context of high βIII-tubulin expression levels. A prior neoadjuvant ixabepilone monotherapy study showed that higher βIII-tubulin mRNA levels were associated with higher pCR rates [24, 27], suggesting that this molecule may be a marker of resistance to paclitaxel but continued sensitivity to ixabepilone. The results of the current study, however, showed that pCR rates were similarly high for both arms, suggesting that βIII-tubulin status defined by IHC should not be used for therapeutic decision making. The increased chemosensitivity of βIII-tubulin-positive tumors in this study may be explained partly by its strong positive association with TNBC and ER-negative phenotype. These cancers differ in many molecular features from ER-positive cancers and are generally more sensitive to chemotherapy [37, 38]; however, long-term outcome for these patients is generally poor [28, 39]. Because long-term follow-up data on DFS and OS were not collected during this study, the potential prognostic value of βIII-tubulin expression reported by others [40] is not possible to ascertain.

The two regimens used in this study were generally well tolerated. PN, commonly associated with microtubule-stabilizing agents [41], occurred at similar rates with both drugs; however, few events were grade 3 (4.1% and 3.5%, respectively), and none were grade 4. Discontinuation due to drug-related PN was uncommon in both arms (3.4% and 4.9%, respectively). Neutropenia was more common with ixabepilone; however, febrile neutropenia remained uncommon (one patient in each arm). Consistent with this favorable tolerability profile, most patients (>80%) were able to complete the planned number of cycles of ixabepilone or paclitaxel.

Conclusion

NC with AC followed by ixabepilone Q3W or weekly paclitaxel was well tolerated with no significant difference in efficacy, as measured by pathologic and clinical response. βIII-Tubulin expression was not significantly associated with a difference in treatment response between ixabepilone and paclitaxel in the neoadjuvant treatment of early stage BC.

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Disclosures

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