A Randomized Phase II Study of Eribulin/Cyclophosphamide or Docetaxel/Cyclophosphamide as Neoadjuvant Therapy in Operable HER2-negative Breast Cancer

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Abstract

The present phase II study compared the combination of eribulin plus cyclophosphamide (ErC) to docetaxel plus cyclophosphamide (TC) as neoadjuvant therapy for HER2$^-$ breast cancer patients. Patients received either eribulin 1.4 mg/m^2 on days 1 and 8 plus cyclophosphamide 600 mg/m^2 on day 1 or docetaxel 75 mg/m^2 plus cyclophosphamide 600 mg/m^2 on day 1 for 6 cycles before surgery. Neoadjuvant ErC showed no greater efficacy than TC.

Background: Eribulin mesylate is a non–taxane microtubule inhibitor effective in the treatment of metastatic breast cancer refractory to anthracyclines and taxanes. In preclinical studies, additional mechanisms of eribulin included reversal of epithelial mesenchymal transition and tumor vascular remodeling. The present study compared the safety and efficacy of eribulin plus cyclophosphamide (ErC) to docetaxel plus cyclophosphamide (TC) as neoadjuvant therapy for operable HER2$^-$ breast cancer. **Patients and Methods:** Women with invasive HER2$^-$ breast adenocarcinoma with no distant metastases were eligible. After a 10-patient safety lead-in, the patients were randomized 2:1 to receive either ErC (eribulin 1.4 mg/m^2 on days 1 and 8 plus cyclophosphamide 600 mg/m^2 on day 1) or TC (docetaxel 75 mg/m^2 plus cyclophosphamide 600 mg/m^2 on day 1) administered every 21 days for 6 cycles, followed by surgery. The pathologic complete response (pCR) rate was the primary endpoint. Tumor samples collected at baseline and at surgery were assayed for select epithelial mesenchymal transition and vascular density markers: E-cadherin, vimentin, and CD31 expression. **Results:** A total of 76 patients were enrolled. Of the 76 patients, 10 received ErC in the lead-in phase and 66 were randomized to ErC (n = 44) or TC (n = 22). The pCR rates with ErC and TC were 13% and 9%, respectively. Both regimens produced frequent neutropenia and peripheral neuropathy. Both regimens increased vascular density as measured by CD31 staining. **Conclusion:** The neoadjuvant regimens of ErC and TC resulted in relatively low pCR rates in this patient population. No unexpected toxicities were observed. Our results also provided no suggestion that ErC is a neoadjuvant treatment with greater efficacy than that of standard regimens.

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Introduction

Neoadjuvant chemotherapy (NAC) is the current standard of care in the treatment of patients with locally advanced or inoperable primary breast cancer. In these patients, NAC improves the rate of successful breast conservation surgery, albeit with a slight increase in local failure rates.[1] The response to NAC provides valuable prognostic information and serves as an in vivo sensitivity assay. The achievement of a pathologic complete response (pCR) has correlated

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with improved disease-free survival (DFS) and overall survival (OS), presumably a surrogate for the eradication of micrometastatic disease.[2-4]

Modeled on adjuvant chemotherapy, NAC regimens commonly include a backbone of anthracyclines and/or taxanes. The combination of doxorubicin and cyclophosphamide (AC), formerly a standard adjuvant regimen, was improved by the addition of taxanes, either concurrently or sequentially.[5,6] In addition, substitution of docetaxel for doxorubicin in combination with cyclophosphamide (TC) demonstrated a 31% improvement in OS compared with AC.[7] Furthermore, the substitution of docetaxel for doxorubicin in combination with cyclophosphamide (TC) reduced cardiac toxicity.[7] Therefore, the substitution of new drugs with potential advantages (increased efficacy or reduced toxicity) remains a valid area of exploration in the treatment of operable breast cancer.

Eribulin mesylate is a unique non−taxane, structurally modified synthetic analogue of halichondrin B that inhibits microtubule dynamics by a novel mechanism of action distinct from other tubulin-targeting agents.[8] Eribulin distinguishes itself from other tubulin-targeting agents by creating an irreversible mitotic blockade, leading to loss of long-term cell viability.[9] Preclinical studies suggested additional mechanisms of action, including reversal of the epithelial to mesenchymal transition (EMT) common in triple-negative breast cancer (TNBC),[10] and remodeling of tumor vasculature.[11]

In the EMBRACE (Eisai Metastatic Breast Cancer Study Assessing Physician's Choice vs. E7389) trial, treatment with eribulin improved OS (vs. investigator-choice chemotherapy) in patients with metastatic breast cancer with disease refractory to anthracyclines and taxanes.[12] A pooled analysis of 2 large randomized trials confirmed the OS benefit of eribulin versus other chemotherapy agents for patients with anthracycline- and taxane-refractory metastatic breast cancer.[13] In the pooled study, particular benefit with eribulin was seen in the subgroup of patients with TNBC. The high activity level of eribulin makes it an ideal candidate for inclusion in regimens for early-stage breast cancer. Eribulin also has a potentially favorable toxicity profile compared with taxanes and avoids the well-known risk of late toxicities associated with anthracyclines. In chemotherapy-naive, early-stage breast cancer patients, it was expected that standard-dose eribulin would be well tolerated when combined with cyclophosphamide.

In the present randomized phase II trial, we substituted eribulin for docetaxel and compared eribulin/cyclophosphamide (ErC) and standard docetaxel/cyclophosphamide (TC) in the neoadjuvant treatment of HER2$^-$ breast cancer. Select markers for EMT (E-cadherin and vimentin) and CD31, an endothelial marker reflecting changes in tumor vasculature, were analyzed in the pre- and post-treatment tumor samples.

Patients and Methods

Study Design

The present study was an open-label, multicenter, randomized phase II trial comparing ErC and TC in the neoadjuvant treatment of operable HER2$^-$ breast cancer. Before beginning the randomized trial, 10 patients were treated with ErC to confirm the safety and feasibility of the combination. Once safety had been confirmed, subsequent patients were stratified by hormone receptor status (positive vs. negative) and randomized in a 2:1 ratio to ErC or TC.

After completion of NAC, the patients underwent definitive breast surgery (mastectomy or lumpectomy). Axillary node sampling (sentinel lymph nodes and/or axillary dissection) accompanied breast surgery, unless it had been performed before NAC. After surgery, patients received local regional radiotherapy and/or adjuvant antiestrogen therapy according to standard guidelines. Tumor samples for exploratory biomarker evaluations were collected when available at baseline and again at surgery in patients with residual invasive disease.

The primary endpoint was the pCR rate. In the present study, pCR was defined as the absence of invasive tumor in the breast and axillary lymph nodes at definitive surgery as determined by the local pathologist. The secondary endpoints included the clinical response rate, 2-year DFS rate, and treatment-related toxicity.

The study was registered with ClinicalTrials.gov (NCT01527487) and conducted according to the ethical principles of the Declaration of Helsinki and in accordance with the International Conference on Harmonisation Guideline for Good Clinical Practice. The institutional review board at each participating site approved the protocol, and all enrolled patients provided written informed consent.

Eligibility

Women with histologically confirmed HER2$^-$ stage cT2-T3, cN0-N2, M0 invasive breast cancer were eligible. Clinical T1 tumors were allowed only if clinical axillary lymph node involvement was present. Axillary lymph node sampling was permitted before surgery; pN3a disease was also allowed. Bilateral synchronous tumors were permitted, provided both tumors were HER2$^-$ and $\geq$ 1 tumor otherwise met the inclusion criteria. HER2$^-$ status was defined as either HER2 immunohistochemical staining of 0 to 1+ or negative fluorescence in situ hybridization/silver in situ hybridization testing (ratio $<$ 2.2).[14] The patients were required to have no evidence of metastatic disease by a complete staging workup completed $\leq$ 8 weeks before initiation of the study treatment. Additional eligibility requirements included age $\geq$ 18 years; Eastern Cooperative Oncology Group performance status $\leq$ 2; and adequate hematologic, renal, and hepatic function.

The exclusion criteria included T4 or inflammatory breast cancer; peripheral neuropathy grade $>$ 1; previous radiotherapy that included $\geq$ 30% of bone marrow; concurrent hormonal therapy with the exception of GnRH agonists; left ventricular ejection fraction $<$ 45% using multigated acquisition scan or echocardiogram; QT interval $>$ 480 ms (Bazett's formula) or use of QT-prolonging drugs.

Pretreatment Evaluation

Before treatment, a complete medical history, physical examination, complete blood count, comprehensive metabolic panel, pregnancy test, peripheral neuropathy assessment, and breast imaging (mammogram, magnetic resonance imaging, or ultrasonography) were required within 45 days of study treatment. Staging evaluations were required $\leq$ 8 weeks before the initiation of chemotherapy and included computed tomography scan of the chest, abdomen, and pelvis and either a bone scan or positron

emission tomography scan. Magnetic resonance imaging or computed tomography of the brain was required only if indicated by the clinical symptoms.

Treatment

The first 10 patients entering the trial were not randomized and all received ErC (eribulin, 1.4 mg/m^2 intravenously [IV] on days 1 and 8; cyclophosphamide, 600 mg/m^2 IV on day 1). After all 10 patients had received $\geq$ 1 cycle of ErC, the safety and feasibility was confirmed. All grade adverse events (AEs), grade 3/4 toxicities, and dose modifications were assessed in the 10 lead-in patients, and no safety signal was observed. Subsequent patients were randomized (2:1) to receive either ErC (same doses) or TC (docetaxel, 75 mg/m^2 IV; cyclophosphamide, 600 mg/m^2 IV; both on day 1). Both regimens were administered every 21 days for 6 cycles, followed by surgery.

Patients received prophylactic antiemetics and premedications according to standard institutional guidelines. With the exception of the first cycle of treatment the for lead-in patients, colony-stimulating growth factor use was allowed in accordance with the American Society of Clinical Oncology guidelines[15] or at the discretion of the treating physician.

After completion of 6 cycles of NAC, the patients were evaluated to determine the response to treatment and eligibility for surgery. The choice of surgical procedure was at the discretion of the treating surgeon. After definitive surgery, the patients received no further chemotherapy. Adjuvant radiotherapy and/or hormonal therapy was administered per standard guidelines.

The patients were evaluated for recurrence every 6 months during the first 5 years after surgery. Patients with documented disease recurrence were followed up every 3 months for survival status. Subsequently, it was decided to limit the follow-up period to 2 years after surgery.

For all efficacy comparisons, the intent-to-treat population was used. Although it is customary to include only the randomized patients in efficacy analyses, given that the lead-in patient toxicity and correlative study comparisons were similar to the population of randomized patients, it was elected to include these lead-in patients in the efficacy evaluations. The demographic and pretreatment characteristics were summarized for all patients using descriptive statistics.

Dose Modifications

The doses of the drugs in both regimens were modified if treatment-related toxicity occurred. Full-dose modification instructions were specified in the study protocol. A maximum of 2 dose reductions was permitted for each agent, and reduced doses were not re-escalated. Dose levels -1 and -2 for eribulin were 1.1 mg/m^2 and 0.7 mg/m^2; for cyclophosphamide were 500 mg/m^2 and 400 mg/m^2; and for docetaxel were 60 mg/m^2 and 45 mg/m^2, respectively. Patients requiring $>$ 2 dose reductions or treatment delays of $>$ 3 weeks were discontinued from study treatment.

Hematologic toxicity was expected to be the major side effect of treatment, and dose modifications were similar for both regimens. Dose modification for nonhematologic toxicity was determined by the toxicity occurring during the preceding treatment cycle and was based on the worst toxicity grade reported. Treatment was discontinued for patients who experienced any grade 4 nonhematologic toxicity or grade 3 neuropathy lasting $\geq$ 7 days. For less severe toxicity, dosing was withheld until toxicity improved to grade $\leq$ 1. If resolution required $>$ 3 weeks, the protocol treatment was discontinued. If toxicity could be attributed to only 1 of the agents, dose reductions involved only the offending agent. Otherwise, the doses of both drugs were reduced. Patients could continue single-agent treatment after discontinuation of the offending agent.

Definition of Response

The clinical response was initially assessed by physical examination (or by imaging studies for patients whose baseline breast tumor or axillary lymph nodes were nonpalpable or difficult to measure by physical examination) after completion of 3 cycles of treatment to remove patients without a response from treatment. On completion of NAC, the response to treatment was assessed using breast imaging. Clinical responses after completion of NAC were defined using the Response Evaluation Criteria In Solid Tumors, version 1.1. The pathologic response to NAC was determined by the pathologists at the participating sites.

Correlative Analysis

Several markers postulated to be affected by treatment with eribulin were assayed in the biopsy specimens at baseline and after NAC. The expression of E-cadherin, vimentin, and CD31 was evaluated using immunochemical (IHC) assays. All assays were performed on 4-mm tissue sections cut from formalin-fixed paraffin-embedded tumor tissue. Ventana Medical Systems reagents were used with Benchmark platform staining. For E-cadherin and vimentin staining, samples were considered positive if $>$ 10% of tumor cells demonstrated cytoplasmic or nuclear labeling. The percentage of tumor cells expressing these proteins was determined, and protein expression was quantified and scored (0, no expression; 1+, weak expression; 2+, moderate expression; 3+, strong expression). The changes in the pre- and post-treatment IHC scores and in the percentage of cells expressing either E-cadherin or vimentin were determined for each pair of biopsy specimens, and the mean values with the standard error of the mean were calculated for each treatment regimen. CD31 protein expression was determined by scanning the slides under 10$\times$ magnification and scoring the number of CD31$^+$ blood vessels in a field of vision as follows: 0, $<$ 10 blood vessels; 1, 10 to 20 blood vessels; and 2, $>$ 20 blood vessels. Changes in this score between the pre- and post-treatment samples, and the mean values were calculated with the standard error of the mean.

Statistical Analysis

A pCR rate of $\geq$ 18% was the response threshold for the present study. The following assumptions were used for sample size calculations: hypothesis H0, pCR $\leq$ 8% against HA: pCR $\geq$ 23%. The type I error rate (α) was set to 10%, and the power ($1 - \beta$) was set to 90%.

Table 1 Patient and Disease Characteristics (n = 76)

Characteristic	ErC (n = 54)	TC (n = 22)	Total (n = 76)
Age, y			
Median	53	51	52
Range	23-77	38-73	23-77
Race			
White	35 (65)	16 (73)	51 (67)
Black/African American	18 (33)	5 (23)	23 (30)
Asian	1 (2)	1 (4)	2 (3)
Baseline ECOG			
0	48 (89)	18 (82)	66 (87)
1	5 (33)	4 (18)	9 (12)
2	1 (2)	0	1 (1)
Histologic type			
Invasive ductal	42 (78)	18 (82)	60 (79)
Invasive lobular	8 (15)	3 (14)	11 (14)
Mixed ductal/lobular	2 (4)	0	2 (3)
Invasive carcinoma, NOS	1 (2)	1 (4)	2 (3)
Invasive papillary	1 (2)	0	1 (1)
Ductal carcinoma in situ	16 (30)	4 (18)	20 (26)
Hormone receptor status			
ER$^+$/PR$^+$	28 (52)	9 (41)	37 (49)
ER$^-$/PR$^-$	19 (35)	6 (27)	25 (33)
ER$^+$/PR$^-$	6 (11)	6 (27)	12 (16)
ER$^-$/PR$^+$	1 (2)	1 (4)	2 (3)
Clinical T stage			
T1	3 (6)	2 (9)	5 (7)
T2	35 (65)	13 (59)	48 (63)
T3	15 (28)	7 (32)	22 (29)
T4a or T4b	1 (2)[a]	0	1 (1)
Clinical axillary node status			
Clinically positive	28 (52)	11 (50)	39 (51)
Clinically negative	26 (48)	11 (50)	37 (49)
Baseline clinical tumor size, cm			
Median	2.8	3.1	3
Range	0.4-10	1.1-9	0.4-10

Data presented as n (%).
Abbreviations: ECOG = Eastern Cooperative Oncology Group; ER = estrogen receptor; ErC = eribulin, cyclophosphamide; NOS = not otherwise specified; PR = progesterone receptor; TC = docetaxel, cyclophosphamide.
[a]This patient had stage T4bN1 at study entry and was not eligible per protocol (T stage $\leq$ 3) but was granted permission to be treated; the exclusion of patients with stage T4a and T4b disease resulted from an oversight in the original protocol design. A planned amendment to include these patients was prepared; however, because trial accrual was near completion at that time, it was not submitted to the Food and Drug Administration or institutional review boards.

Using these parameters and a 2:1 randomization, the trial required 39 evaluable patients receiving ErC and 20 patients receiving TC. To account for an estimated 10% nonevaluable rate, 66 patients (44 in arm 1 and 22 in arm 2) were to be randomized.

The DFS and OS estimates were calculated using the Kaplan-Meier method. Toxicity was graded using the National Cancer Institute Common Toxicity Criteria, version 4.0, and was evaluated in all patients who had received $\geq$ 1 dose of study treatment. For the correlative analyses, the *P* values were calculated, and the treatment arms compared using the Wilcoxon signed ranks test (nonparametric paired *t* test).

Results

Patient Characteristics

From July 2012 to March 2014, 76 patients were enrolled in the study (Table 1). The median age was 52 years (range, 23-77 years), and 51 patients (67%) were estrogen receptor-positive (ER$^+$) and/or progesterone receptor (PR$^+$). Most patients (79%) had infiltrating ductal adenocarcinoma, 14% had invasive lobular adenocarcinoma, and 7% had other or combined histologic findings. The median clinical tumor size was 3 cm (range, 0.4-10 cm), with clinical axillary lymph node involvement in 51%. The major prognostic factors were well balanced between the 2 groups (Table 1).

Figure 1 CONSORT (Consolidated Standards of Reporting Trials) Diagram of Patients

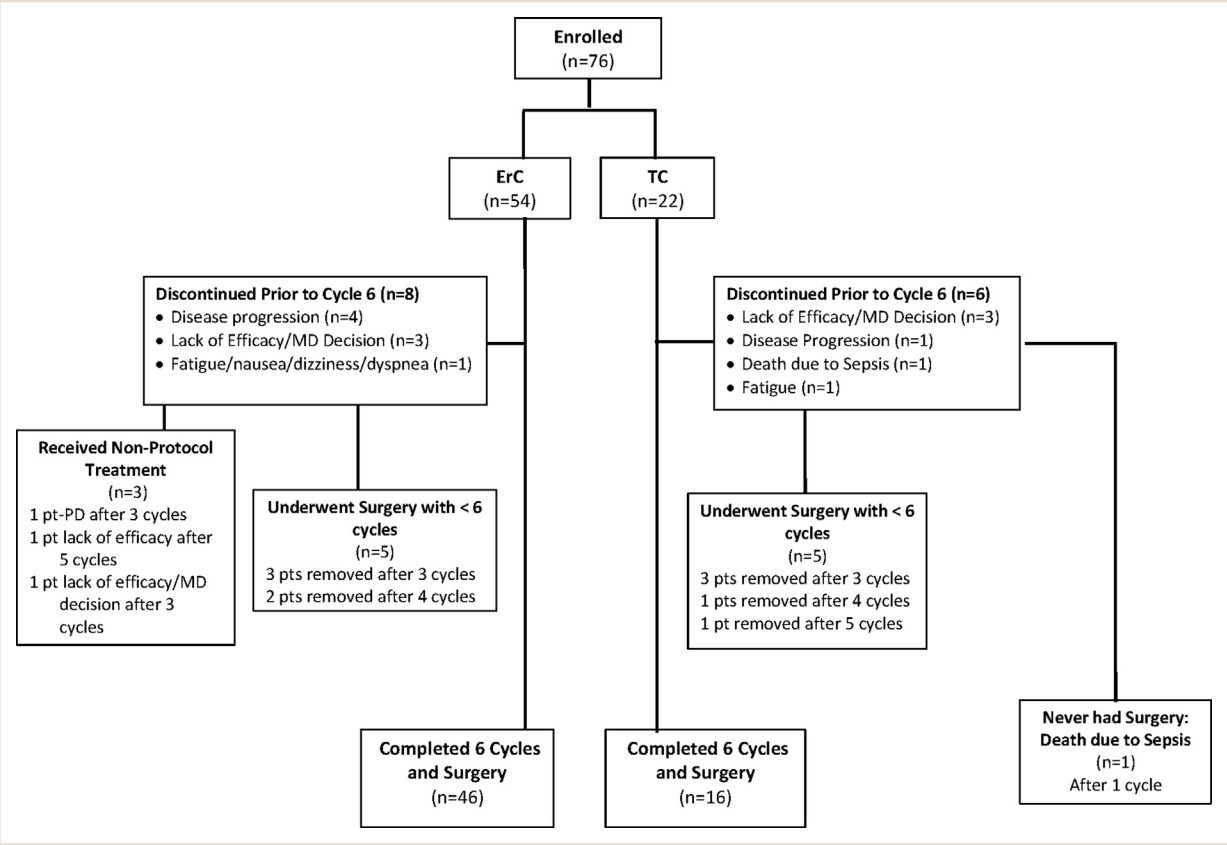
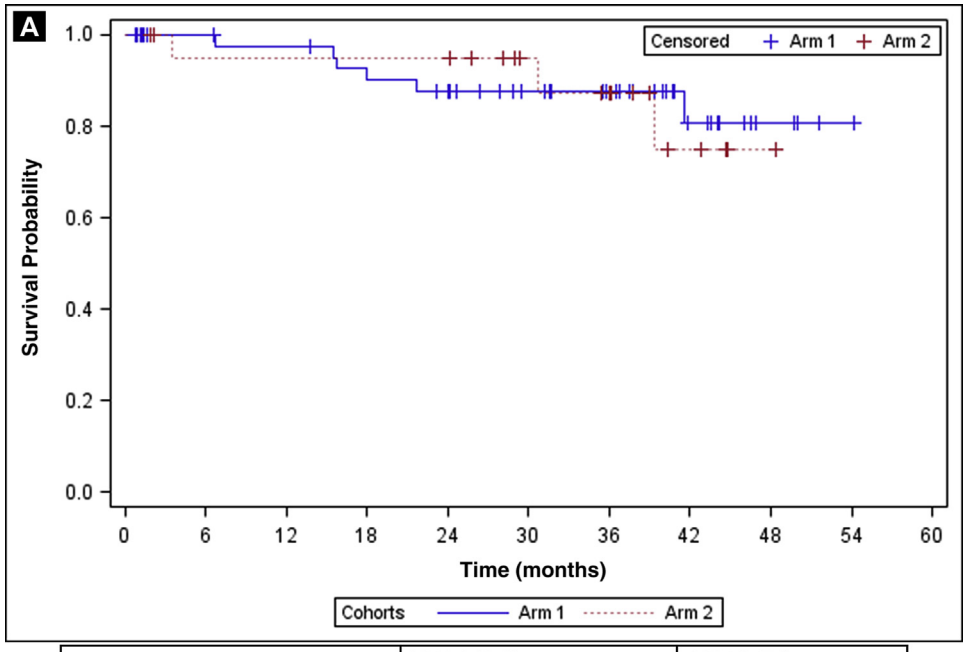


Table 2 Response to Treatment (n = 76)

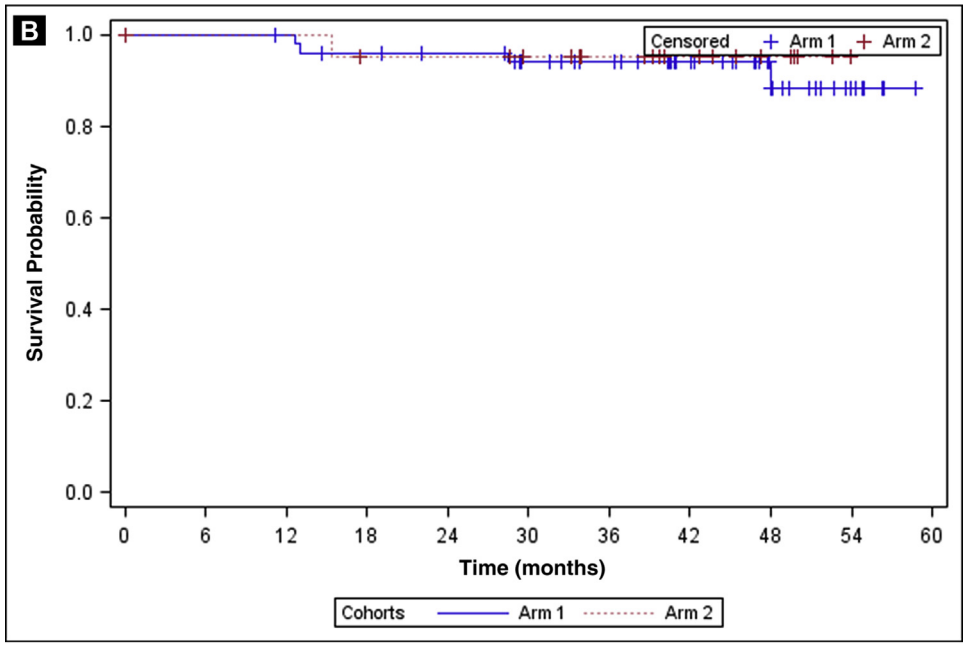
Response Assessment	ErC (n = 54)	TC (n = 22)	Total (n = 76)
Pathologic response			
pCR	7 (13)	2 (9)	9 (12)
ER^+ and/or PR^+	3/35 (9)	1/16 (6)	4/51 (8)
ER^-/PR^-	4/19 (21)	1/6 (17)	5/25 (20)
Residual disease			
Breast only	21 (39)	7 (32)	28 (37)
Lymph node only	0 (0)	1 (4)	1 (1)
Both breast and lymph node	21 (39)	11 (50)	32 (42)
Progressive disease	2 (4)	0	2 (3)
No surgery	3 (6)	1 (4)	4 (5)
Clinical response			
Complete response	9 (17)	1 (4)	10 (13)
Partial response	23 (42)	12 (55)	35 (46)
Stable disease	15 (28)	7 (32)	22 (29)
Progressive disease	7 (13)	1 (4)	8 (11)
Not evaluable	0	1 (4)	1 (1)

Data presented as n (%).
Abbreviations: ER = estrogen receptor; ErC = eribulin, cyclophosphamide; PR = progesterone receptor; TC = docetaxel, cyclophosphamide.

Figure 2 (A) Disease-Free Survival for Patients Who Underwent Surgery (n = 72). (B) Overall Survival (n = 76)



	ARM 1 (ErC)	ARM 2 (TC)
Sample Size	51	21
Median DFS in months (95% CI)	Not reached (Not reached, Not reached)	Not reached (39.4, Not reached)
24-month DFS probability (95% CI)	88 (73, 95)	95 (68, 99)



	ARM 1 (ErC)	ARM 2 (TC)
Sample Size	54	22
Median OS in months (95% CI)	Not reached	Not reached
24-month OS probability (95% CI)	96 (86, 99)	95 (71, 99)

Table 3 Results of Correlative Analyses[a] (n = 40)

Treatment Arm	E-cadherin IHC Score		Cells Expressing E-cadherin, %		Vimentin IHC Score		Cells Expressing Vimentin, %		CD31 Vessel Density	
	Before	After	Before	After	Before	After	Before	After	Before	After
ErC										
Mean	2	1.783	68	68	1.033	0.87	26	22	0.233	0.867
SEM	0.161	0.187	5	6	0.255	0.232	7	6	0.079	0.133
Difference, %	89.20		99.80		83.90		85.70		371.40	
TC										
Mean	1.944	1.389	69	57	1.056	0.889	23	27	0.111	0.556
SEM	0.317	0.38	10	14	0.429	0.398	10	13	0.111	0.242
Difference, %	71.40		82.30		84.20		114.30		500.00	

Abbreviations: After = after treatment; Before = before treatment; ER = estrogen receptor; ErC = eribulin, cyclophosphamide; PR = progesterone receptor; TC = docetaxel, cyclophosphamide.
[a]*P* values for post-treatment versus pre-treatment levels (Wilcoxon signed ranks test [nonparametric paired *t* test])—ErC arm: E-cadherin IHC scores, $P = .152$; percentage of cells expressing E-cadherin, $P = .844$; vimentin IHC scores, $P = .490$; percentage of cells expressing vimentin, $P = .551$; CD31 vessel density scores, $P = .001$; TC arm: E-cadherin IHC scores, $P = .125$; percentage of cells expressing E-cadherin, $P = .5$; vimentin IHC scores, $P = 5$; percentage of cells expressing vimentin, $P = 1.00$; CD31 vessel density scores, $P = .125$.

Treatment Received

The study treatment received by the 76 patients in the present study is summarized in Figure 1. Of the 76 patients, 62 (82%) completed 6 cycles of NAC and underwent definitive surgery (ErC, 46 patients [85%]; TC, 16 patients [73%]). An additional 10 patients received < 6 cycles of NAC (5 patients in each regimen) but underwent definitive surgery per protocol. Eight patients received < 6 cycles of NAC because of disease progression and/or lack of efficacy, and 2 patients discontinued NAC because of toxicity. Of the 72 patients who had undergone surgery per protocol, 57 (79%) underwent mastectomy and 15 (21%) underwent breast conservation surgery. Four patients discontinued NAC early

Table 4 Treatment-related Adverse Events

CTCAE Term	ErC (n = 54)		TC (n = 22)		Overall (n = 76)
	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	
Hematologic AEs ≥ 5%					
Neutropenia	15 (28)	12 (22)	1 (5)	4 (18)[a]	32 (42)
Febrile neutropenia	1 (2)	3 (6)	0 (0)	1 (5)	5 (7)
Anemia	18 (33)	0 (0)	8 (36)	0 (0)	26 (34)
Leukopenia	10 (19)	5 (9)	1 (5)	4 (18)	20 (26)
Thrombocytopenia	3 (6)	0 (0)	1 (5)	0 (0)	4 (5)
Nonhematologic AEs ≥ 10%					
Fatigue	39 (72)	1 (2)	13 (59)	1 (5)	54 (71)
Alopecia	32 (59)	0 (0)	14 (64)	0 (0)	46 (61)
Nausea	33 (61)	0 (0)	8 (36)	0 (0)	41 (54)
Peripheral neuropathy	19 (35)	0 (0)	7 (32)	3 (14)	29 (38)
Constipation	16 (30)	0 (0)	6 (27)	0 (0)	22 (29)
Diarrhea	12 (22)	0 (0)	10 (45)	0 (0)	22 (29)
Headache	11 (20)	0 (0)	4 (18)	0 (0)	15 (20)
Myalgia	9 (17)	0 (0)	5 (23)	1 (5)	15 (20)
Hot flashes	8 (15)	1 (2)	3 (14)	0 (0)	12 (16)
Edema	7 (13)	0 (0)	4 (18)	0 (0)	11 (15)
Mucositis	3 (6)	0 (0)	8 (36)	0 (0)	11 (15)
Arthralgia	6 (11)	0 (0)	5 (23)	1 (5)	10 (13)
Dysgeusia	2 (4)	0 (0)	7 (32)	0 (0)	10 (13)
Dyspepsia	9 (17)	0 (0)	0 (0)	0 (0)	9 (12)
Insomnia	5 (9)	0 (0)	3 (14)	0 (0)	8 (11)

Data presented as n (%).
Abbreviations: AEs = adverse events; CTCAE = Common Terminology Criteria for Adverse Events; ErC = eribulin, cyclophosphamide; TC = docetaxel, cyclophosphamide.
[a]One patient developed grade 5 neutropenic sepsis.

and had either received no further treatment (1 death from treatment-related toxicity) or had received treatment off protocol (3 patients).

The median number of treatment cycles delivered in both arms was 6 (ErC, range, 3-6 cycles; TC, range, 1-6 cycles). Thirteen ErC-treated patients (24%) required dose reduction of eribulin, including 1 patient who required 2 dose reductions. Five TC patients (23%) required docetaxel dose reduction, including 2 patients, who required 2 dose reductions. Most dose reductions were caused by either neutropenia or peripheral neuropathy. Growth factor usage differed between the 2 treatment arms, with 24% of patients in the ErC arm using prophylactic growth factors compared with 91% of patients in the TC arm.

Efficacy

The pathologic responses to treatment are summarized in Table 2. Of the 72 patients who had undergone definitive surgery, 9 patients (12%) achieved a pCR (ErC, 7 [13%]; TC, 2 [9%]). Of the 9 patients with a pCR, 8 completed all 6 planned cycles of NAC and 1 completed 4 cycles. Of the 25 patients with TNBC, 5 patients achieved a pCR (20%; ErC, 21%; TC, 17%) compared with 4 of 51 patients (8%) with hormone receptor-positive tumors. Twenty-eight patients (37%) had residual tumor in the breast only (ErC, 21 [39%]; TC, 7 [32%]). One TC patient had residual disease only in the axillary lymph nodes. Thirty-two patients (42%) had residual disease in the breast and lymph nodes both (ErC, 21 [39%]; TC, 11 [50%]). One patient in the TC arm (4%) and 3 patients in the ErC arm (6%) did not undergo surgery.

Ten patients (13%) had clinical complete responses before surgery (ErC, 9 [17%]; TC, 1 [5%]). An additional 35 patients (46%) had partial clinical responses (ErC, 23 [43%]; TC, 12 [55%]). The clinical response was not predictive of a pCR.

After a median follow-up of 3.5 years, the median DFS was not reached for either treatment. The 24-month DFS rates for ErC and TC were 88% and 95%, respectively (Figure 2A). The estimated 24-month OS rates for ErC and TC were 96% and 95%, respectively (Figure 2B).

Correlative Analysis With EMT

Pre- and post-treatment tissue samples were requested from all patients. However, only single samples were obtained from 29 patients, and inadequate tissue samples were submitted by 7 patients. Therefore, paired samples were collected from 31 patients treated with ErC and 9 patients treated with TC. The results of testing for the markers of EMT (E-cadherin and vimentin) and vascular density (CD31) are summarized in Table 3. No significant changes were found in E-cadherin and vimentin expression after neoadjuvant ErC or TC as measured using either the IHC score or the percentage of cells expressing these proteins. Vascular density, as measured by CD31, was increased after treatment with either regimen. A comparison of the 2 regimens was not possible owing to the small patient numbers.

Toxicity

Treatment-related toxicities (all grades) are listed in Table 4. Neutropenia was the most common severe (grade 3/4) toxicity and occurred in 21% of the patients (ErC, 22%; TC, 18%). However, only 5 episodes of febrile neutropenia occurred (ErC, 4; TC, 1), including 1 fatal episode (TC arm). Peripheral neuropathy was the major nonhematologic toxicity. Neuropathy grade > 1 occurred in 35% and 46% of the ErC and TC patients, respectively. Grade 3/4 neuropathy occurred in 3 patients (14%) receiving TC and in no patient receiving ErC. Mucositis, arthralgia, and diarrhea (most episodes were grade 1/2) were numerically more frequent with TC, and nausea (grade 1/2) was more common with ErC.

Hospitalizations were required for 3 patients for febrile neutropenia and for 1 patient for fatal sepsis associated with neutropenia. The patient with fatal sepsis had not received prophylactic granulocyte colony-stimulating factor.

Discussion

NAC for early-stage breast cancer results in DFS and OS outcomes similar to those achieved with adjuvant therapy.[1] Achieving a pCR with NAC has correlated with improved DFS and serves as a surrogate marker of treatment efficacy. In the past, regimens of increased efficacy have been developed by substituting or adding chemotherapy agents with single-agent efficacy advantages compared with older agents. With the proven efficacy of eribulin in metastatic breast cancer patients with disease refractory to anthracyclines and taxanes, we postulated that this nonanthracycline combination with cyclophosphamide would be tolerated, effective, and safe. This was predicated from the results of the pivotal USOR 9735 study of TC for 4 cycles versus AC for 4 cycles, which showed overall superiority for TC, establishing this combination as an effective nonanthracycline regimen.[7] In a subsequent collaboration between US Oncology Research and the National Surgical Adjuvant Breast and Bowel Project, a joint analysis of 3 independent clinical trials known as the ABC (anthracyclines in early breast cancer) trials compared the 2-drug TC combination with a 3-drug taxane plus anthracycline arm, and no difference was seen in OS but there was a small absolute benefit of 2.5% in the 4-year DFS for the taxane plus anthracycline arm, albeit accompanied by several cases of acute leukemia.[16] Exploratory subgroup analyses suggested that the taxane plus anthracycline regimen provided no benefit in the ER$^+$, node negative group and may provide minimal benefit in ER$^+$ patients with 1 to 3 lymph nodes.[16] Given the heterogeneity of breast cancer, additional follow-up and correlative studies to identify biomarkers are critical to fully determine the utility of anthracyclines in breast cancer.

In the present trial, we first established the feasibility and safety of the ErC regimen, using a standard dose of eribulin (1.4 mg/m^2 on days 1 and 8) combined with a standard dose of cyclophosphamide (600 mg/m^2 on day 1), both given in 21-day cycles. We then performed a randomized phase II trial comparing ErC with a standard TC regimen. However, the pCR rates were low with both regimens: 13% with ErC and 9% with TC. The modest pCR rates might have partially been because 67% of the patients treated in our neoadjuvant trial had hormone receptor-positive tumors, a phenotype that is well-recognized for its low pCR rates with NAC. The regimens in the present trial produced a combined 8% pCR rate in hormone receptor-positive tumors. However, the overall pCR rate of 20% in patients with TNBC was also relatively low and does not suggest any therapeutic advantage for ErC compared with other standard neoadjuvant regimens.

Although no efficacy benefit was demonstrated, 6 cycles of the ErC regimen proved to be feasible to administer as NAC. Neutropenia and neuropathy were common, although severe neuropathy did not occur with ErC. The toxicity profiles also suggest that ErC was better tolerated than TC with respect to mucositis, arthralgia, and diarrhea.

Eribulin demonstrated an OS advantage in patients with anthracycline- and taxane-refractory advanced breast cancer. It has been postulated that this survival benefit might be attributed to eribulin's potential to suppress new metastases through its effects on the EMT pathway. Preclinical studies demonstrated that eribulin had an effect in reversing the EMT that accompanies aggressive tumor phenotypes such as TNBC.[11] In addition, eribulin induced tumor vascular remodeling, resulting in increased tumor perfusion, increased microvessel density, and decreased vascular endothelial growth factor expression.[12] As a part of the present study, we evaluated several biomarkers as potential correlates and predictors of eribulin efficacy. EMT activation is accompanied by loss of the epithelial marker E-cadherin and the increased expression of mesenchymal markers such as N-cadherin and vimentin. Using pre- and post-treatment tumor biopsy samples, we compared the effects of the 2 treatment regimens on the markers of EMT (E-cadherin, vimentin) and tumor vascular remodeling (CD31). EMT is important in erubilin's mechanism of action, and we expected to see increased E-cadherin and decreased vimentin levels after treatment, with increased CD31 expression.

Although the patient numbers were small (ErC, 31; TC, 9), neither regimen produced changes in E-cadherin or vimentin when pre- and post-treatment levels were assayed. Both regimens produced marked increases in the CD31 vessel density scores. The small number of patients precluded meaningful comparisons of the 2 regimens. Therefore, these findings do not support an important role for reversal of EMT by eribulin and could not differentiate the 2 regimens in terms of the induction of tumor vascular remodeling. The small number of patients with a pCR did not allow correlation of the biomarker changes with the treatment response.

To the best of our knowledge, the present study is the first community-based study evaluating eribulin combined with cyclophosphamide for breast cancer. We attempted to validate the preclinical data of erubulin's role in affecting the EMT pathway.

One limitation of the present study was that it was not initially designed nor sufficiently powered to separately analyze the TNBC and ER^+ patient populations. The inability to collect paired tissue biopsies from all treated patients limited our ability to draw definite conclusions from the biomarker analysis.

Conclusion

NAC with ErC produced a pCR rate of 13%, similar to results achieved with other standard 2-drug regimens. The findings from the biomarker studies did not suggest substantial differences between the ErC and TC regimens in terms of EMT reversal or effects on tumor vascular remodeling. Further development of the ErC regimen as neoadjuvant treatment for operable breast cancer is not recommended.

Clinical Practice Points

- The combination of ErC was not more efficacious than TC as NAC for $HER2^-$ breast cancer patients.
- The toxicity profiles were similar for these treatment regimens, with both regimens producing frequent neutropenia and peripheral neuropathy.
- Biomarker studies of E-cadherin, vimentin, and CD31 expression did not suggest substantial differences between the ErC and TC regimens in terms of EMT reversal or effects on tumor vascular remodeling.
- Further development of the ErC regimen as neoadjuvant treatment for operable breast cancer is not recommended.

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Disclosure

The authors declare that they have no competing interests.

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