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ORIGINAL REPORT

Adjuvant Cyclophosphamide and Docetaxel With or Without Epirubicin for Early *TOP2A*-Normal Breast Cancer: DBCG 07-READ, an Open-Label, Phase III, Randomized Trial

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ABSTRACT

Purpose

Administration of anthracycline and taxane therapy in the adjuvant setting is considered a standard for breast cancer. We evaluated a non–anthracycline-based regimen in *TOP2A*-normal patients.

Patients and Methods

In this multicenter, open-label, phase III trial, 2,012 women with early *TOP2A*-normal breast cancer and at least one high-risk factor were randomly assigned to receive six cycles of docetaxel (75 mg/m^2) and cyclophosphamide (600 mg/m^2) every 3 weeks (DC) or three cycles of epirubicin (90 mg/m^2) and cyclophosphamide (600 mg/m^2) followed by three cycles of docetaxel (100 mg/m^2; EC-D). The primary end point was disease-free survival (DFS) after a median of 5 years of follow-up. Secondary end points were patient-reported toxicity, overall survival (OS), and distant disease–free survival.

Results

At a median estimated potential follow-up of 69 months, 5-year DFS was 87.9% (95% CI, 85.6% to 89.8%) in the EC-D arm and 88.3% (95% CI, 86.1% to 90.1%) in the DC arm. There was no significant difference in the risk of DFS events (hazard ratio [HR], 1.00; 95% CI, 0.78 to 1.28; P = 1.00), distant disease–free survival (HR, 1.12; 95% CI, 0.86 to 1.47; P = .40), or mortality (HR, 1.15; 95% CI, 0.83 to 1.59; P = .41) in the intent-to-treat analysis. A significant interaction between menopausal status and treatment group was observed for DFS (P = .04) but not for OS (P = .07). Patients with grade 3 tumors derived most benefit from DC, and patients with grade 1 to 2 tumors derived most benefit from EC-D (DFS: interaction P = .02; and OS: interaction P = .03). Patients receiving EC-D reported significantly more stomatitis, myalgia or arthralgia, vomiting, nausea, fatigue, and peripheral neuropathy, whereas edema was more frequent after DC.

Conclusion

This study provides evidence to support no overall outcome benefit from adjuvant anthracyclines in patients with early *TOP2A*-normal breast cancer.

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INTRODUCTION

For the past 40 years, adjuvant chemotherapy has improved the survival of an increasing proportion of patients with early breast cancer. The meta-analyses of the Early Breast Cancer Trialists' Collaborative Group have shown that the benefit of cyclophosphamide, methotrexate, and fluorouracil (CMF) was largely independent of patient and tumor characteristics as well as concomitant tamoxifen and that an incremental benefit was obtained from adding an anthracycline to CMF, from substituting methotrexate with doxorubicin or epirubicin, and from administering taxanes concurrently or in sequence with anthracyclines.[1,2]

On average, the benefit achieved by anthracyclines has only been modest, and evidence has suggested that molecular characteristics of the tumor are essential. Overexpression and amplification of human epidermal growth factor receptor 2 (HER2) was retrospectively associated with a significant benefit from anthracyclines in National Surgical Adjuvant Breast and Bowel Project trials B11 and MA.5.[3,4] Only patients with *HER2*-normal breast cancer were included in the

ASSOCIATED CONTENT

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Appendix
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Anthracyclines in Early Breast Cancer (ABC) trials and seemed to derive some benefits from anthracyclines because the joint analysis was unable to demonstrate noninferiority of adjuvant docetaxel and cyclophosphamide (DC) compared with doxorubicin and cyclophosphamide followed by paclitaxel.[5]

TOP2A encodes topoisomerase IIα, an essential enzyme resolving topologic DNA constraints and a direct target of anthracyclines.[6,7] An association between alterations in copy number of *TOP2A* and incremental benefit from substituting methotrexate in CMF with epirubicin was demonstrated in the Danish Breast Cancer Cooperative Group (DBCG) 89D trial.[8,9] A pooled analysis of the DBCG 89D trial together with four additional phase III trials confirmed a greater benefit of anthracyclines in patients with *TOP2A* alterations and a trend toward greater benefit in patients with *HER2*-amplified tumors.[9] Provided that benefit of anthracyclines is largely confined to the subgroup of patients with *TOP2A*-altered tumors, then patients with *TOP2A*-normal tumors could potentially be spared from anthracyclines as part of their adjuvant chemotherapy. The DBCG 07-READ (randomized trial of epirubicin and cyclophosphamide followed by docetaxel against docetaxel and cyclophosphamide) trial compared six cycles of DC with three cycles of epirubicin and cyclophosphamide followed by three cycles of docetaxel (EC-D) in patients with *TOP2A*-normal tumors.

PATIENTS AND METHODS

DBCG 07-READ (ClinicalTrials.gov identifier: NCT00689156) was a nationwide, multicenter, open-label, two-arm, phase III, randomized trial. DBCG coordinated the trial and was responsible for study design, random assignment, collection and management of data, analysis of data, and reporting.[10] The National Danish Ethics Committee (H-D-2008-009) approved the trial, and all participants provided written, informed consent before enrollment. The study was conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki.

Eligible patients included node-positive and node-negative high-risk patients (age younger than 39 years, tumor size $>$ 20 mm, grade 2 or 3 ductal carcinoma, estrogen receptor [ER] negative [$<$ 10% positive], and/or HER2 positive) after complete resection of a *TOP2A*-normal invasive carcinoma.[11] Exclusion criteria included the following: pregnancy or breastfeeding; Charlson comorbidity index (CCI) $>$ 2; earlier medical cancer treatment; treatment with a nonapproved product within 30 days; bilateral, locally advanced, or distant disease; and prior malignant disease within 5 years. Patients with any HER2 status were eligible.

Procedures

Tumor blocks were submitted to one of the three regional pathology laboratories, and two invasive tissue cores were transferred to a recipient tissue microarray block, as previously described.[12] *TOP2A* normal was defined as a signal-to-centromere 17 ratio of 0.8 to 1.9 by fluorescent in situ hybridization (TOP2A pharmDX; Dako A/S, Glostrup, Denmark) as specified in the US Food and Drug Administration approval (Code K5333) and otherwise as altered.[11]

A customized version of DBCG's Clinical Data Management System was used for random 1:1 assignment using permuted blocks stratified by institution. Eligible patients with no comorbidity (CCI = 0) were assigned to EC-D (three cycles of epirubicin 90 mg/m^2 and cyclophosphamide 600 mg/m^2, then three cycles of docetaxel 100 mg/m^2) or six cycles of DC (docetaxel 75 mg/m^2 and cyclophosphamide 600 mg/m^2) both given intravenously on day 1 every 3 weeks. In patients with a CCI of 1 or 2, drug doses were reduced initially (epirubicin 60 mg/m^2, cyclophosphamide 500 mg/m^2, then docetaxel 75 mg/m^2 in EC-D arm or docetaxel 60 mg/m^2 and cyclophosphamide 500 mg/m^2 in DC arm). In both groups, docetaxel was accompanied by colony-stimulating factor.

Chemotherapy doses were adjusted according to WBC and platelet counts on day 1 of the scheduled cycle as follows: platelets $>$ 100 $\times$ 10^9/L and WBC $>$ 3.5 $\times$ 10^9/L, 100% of drugs; and platelets 75 to 100 $\times$ 10^9/L or WBC 2.0 to 3.5 $\times$ 10^9/L, 50% of all three drugs. For platelets $<$ 75 $\times$ 10^9/L or WBC $<$ 2.0 $\times$ 10^9/L, the treatment was delayed for 1 week. Patient-reported outcomes were collected by questionnaires before the start of chemotherapy (baseline), on day 20 after each of six cycles, and 6 months after the sixth cycle. The questionnaire was built on a Danish translation of the National Cancer Institute Common Toxicity Criteria version 2.0 and allowed patients to rank predefined adverse effects from 0 (none) to 4 (functional impact).

In node-negative patients, radiotherapy was administered to the residual breast after breast-conserving surgery, and in node-positive (macrometastatic) patients, radiotherapy was administered to regional nodes and residual breast or chest wall at completion of chemotherapy. Ovarian suppression was not allowed, and patients with hormone receptor–positive tumors were allocated to tamoxifen 20 mg per day (if premenopausal) or letrozole 2.5 mg per day (if postmenopausal) for 5 years. Patients with HER2-positive tumors were given trastuzumab intravenously every 3 weeks initiated at cycle 4 and continued for 17 cycles.

Physical examinations were performed at baseline before administration of chemotherapy, at 3 and 6 months after completion of chemotherapy, every 6 months during years 2 to 5, and yearly during years 5 to 10. Mammograms were done every second year in women age 50 years or older and annually in younger women. Additional biochemical tests and imaging were done when indicated by symptoms or signs. Clinical follow-up was continued until first event or a maximum of 10 years.

A complete follow-up on vital status was obtained until October 1, 2016, for all patients through linkage to the Danish Central Population Registry. After completion of clinical follow-up, secondary malignancies were obtained through linkage to the Danish Cancer Registry and the Danish Patient Registry.

Outcomes

The primary end point was disease-free survival (DFS), which was defined as time from random assignment to any first event of invasive ipsilateral or contralateral breast recurrence, local or regional invasive recurrence, distant recurrence, second (nonbreast) invasive cancer, or death from any cause. Secondary end points included overall survival (OS) and distant disease–free survival (DDFS). OS was defined as time from random assignment until death from any cause. DDFS was defined as time from random assignment to distant recurrence, death from any cause, or second (nonbreast) invasive cancer, whereas other first events were considered competing risk events.

Statistical Analysis

The statistical power was estimated under the following assumptions. According to the DBCG database, the expected 3-year DFS rate was 82% after EC-D. Approximately 1,150 patients would be eligible annually for *TOP2A* testing, and 920 (85%) would, according to DBCG 89D, have a *TOP2A*-normal tumor. Approximately 70% of patients were expected to consent to participate. With a recruitment of 1,910 patients in 3 years, after 5 years of follow-up, the study would have 80% power (P = .05 significance level) to detect a 36% improvement with DC compared with EC-D.[8,10]

Follow-up time was quantified in terms of a Kaplan-Meier estimate of potential follow-up. OS and DFS were analyzed unadjusted using the Kaplan-Meier method, and groups were compared using the log-rank test. Unadjusted hazard ratios (HRs) were estimated from the Cox proportional hazards regression model to quantify the effect of treatment regimen. Multivariate Cox proportional hazards models were applied to explore interactions (Appendix Table A1, online only). Multiple fractional polynomial was used for model building.[13] Interactions between treatment and

the covariates were investigated in separate models. The assumptions of proportional hazards were assessed by Schoenfeld residuals and by including a time-dependent component in the model. For competing risk analysis, the Fine-Gray subdistribution hazard model was used. The intent-to-treat (ITT) population was used for analysis of DFS, OS, and DDFS. Dose-intensities and adverse event frequencies were calculated for the per-protocol population, censoring at any crossover to another treatment regimen. For each regimen, cycle, and drug combination, mean relative dose-intensities were calculated. Associations between regimen and other characteristics (excluding unknowns) were analyzed by the χ^2 and Wilcoxon tests. *P* values are two-tailed. Statistical analyses were performed using Stata version 14.2 (STATA, College Station, TX) and SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Between June 2008 and December 2012, after diagnostic workup and definitive breast surgery, 7,086 Danish women were eligible for *TOP2A* screening. A central *TOP2A* analysis was carried out in 5,153 patients, and 4,318 patients (84%) had a *TOP2A*-normal tumor, whereas 835 (16%) had a *TOP2A*-altered tumor. Between July 2008 and December 2012, 12 DBCG centers randomly assigned 2,012 eligible patients to EC-D (n = 1,001) or DC (n = 1,011; Fig 1). One patient in the EC-D group and three patients in the DC group emigrated and were censored between 517 and 1,772 days after random assignment. Complete follow-up for survival was achieved for the remaining 2,008 patients. The random assignment was well balanced, with no significant differences between the EC-D and DC groups (Table 1).

Among the 2,012 patients, two patients from the EC-D arm withdrew consent and were untreated, and in both groups, five patients self-selected the other arm and crossed over. The mean relative dose-intensity (actual/planned mg/m^2) changed similarly in cycles 1 to 3 and overall in cycles 4 to 6 (Fig 2), whereas more dose reductions in cycle 6 were performed in the ED-C group (0.86) compared with the DC group (0.89; $P < .01$). Among the 1,440 patients with ER-positive disease, 18 (1%) eluded endocrine treatment. One of 222 patients with HER2-positive disease declined trastuzumab, and among the 1,780 patients recommended adjuvant radiotherapy, 17 declined, whereas the data on 52 patients were missing.

Study Outcome

This analysis was conducted 5 years after closure of recruitment. Median estimated potential follow-up was 5 years and 9 months for DFS and 5 years and 11 months for OS. A total of 253 first events were observed (Table 2). Figure 3A shows the Kaplan-Meier curves for DFS. In the ITT analysis (n = 2,012), there was no difference in DFS between the EC-D group and the DC group (overall unadjusted HR, 1.00; 95% CI, 0.78 to 1.28; $P = 1.00$). Five-year DFS rates were 87.9% (95% CI, 85.6% to 89.8%) for the EC-D group and 88.3% (95% CI, 86.1% to 90.1%) for the DC group. There was no significant difference in DDFS between the EC-D and DC groups (unadjusted HR, 1.12; 95% CI, 0.86 to 1.47; $P = .40$).

Table 2 lists the causes of death. There is no identifiable pattern behind the numerical difference of 78 deaths in the DC group compared with 68 deaths in EC-D group, and Figure 3B illustrates that there was no statistical difference in mortality (HR, 1.15; 95% CI, 0.83 to 1.59; $P = .41$). Five-year OS rates were 94.8% (95% CI, 93.2% to 96.0%) in the EC-D group and 93.9% (95% CI, 92.2% to 95.3%) in the DC group. One patient died of acute myocardial infarction after the third cycle of DC, which was reported by the investigator as unrelated, and there were no other deaths during or within 30 days of chemotherapy. One patient in the ED-C group and two patients in DC group developed

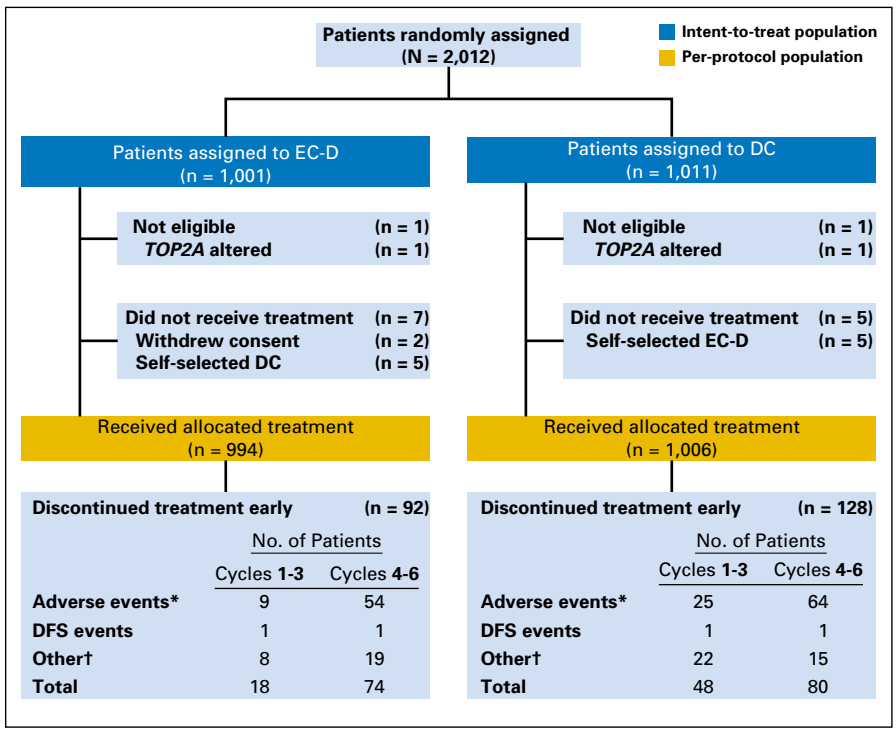

Fig 1. Trial profile of the Danish Breast Cancer Group 07-READ trial. (*) Patient-reported grade 3 or 4 adverse events. (†) Including hypersensitivity, infection, neutropenia, and patient and treating physician's choice. DC, docetaxel and cyclophosphamide; DFS, disease-free survival; EC-D, epirubicin and cyclophosphamide followed by docetaxel; READ, randomized trial of epirubicin and cyclophosphamide followed by docetaxel against docetaxel and cyclophosphamide.

Table 1. Baseline Demographic and Clinical Characteristics of Patients Randomly Assigned and Not Randomly Assigned

Characteristic	No. of Patients (%)		
	EC-D (n = 1,001)	DC (n = 1,011)	Not Randomly Assigned (n = 2,308)
Age at enrollment, years			
< 40	77 (8)	93 (9)	207 (9)
40-44	128 (13)	106 (10)	238 (10)
45-49	199 (20)	204 (20)	433 (19)
50-54	230 (23)	277 (27)	499 (22)
55-59	265 (26)	237 (23)	601 (26)
60-74	102 (10)	94 (9)	330 (14)
Menopausal status			
Premenopausal	508 (51)	544 (54)	1,105 (48)
Postmenopausal	493 (49)	467 (46)	1,203 (52)
Charlson comorbidity index			
0	900 (90)	926 (92)	1,900 (82)
1	82 (8)	68 (7)	282 (12)
2	19 (2)	17 (2)	91 (4)
3+	0	0	35 (2)
Nodal status			
Negative	448 (45)	467 (46)	1,089 (47)
1-3 positive	406 (41)	409 (40)	878 (38)
4-9 positive	106 (11)	101 (10)	235 (10)
> 9 positive	41 (4)	34 (3)	106 (5)
Tumor size, mm			
0-10	131 (13)	127 (13)	359 (16)
11-20	487 (49)	452 (45)	1,047 (45)
21-50	361 (36)	414 (41)	835 (36)
> 50	22 (2)	18 (2)	67 (3)
Histologic type			
Infiltrating ductal carcinoma	873 (87)	871 (86)	2,013 (87)
Infiltrating lobular carcinoma	72 (7)	87 (9)	150 (7)
Other or unknown carcinomas	56 (6)	53 (5)	145 (6)
Malignancy grade (ductal and lobular carcinomas)			
1	159 (16)	176 (17)	364 (16)
2	453 (45)	459 (45)	1,028 (45)
3	328 (33)	311 (31)	759 (33)
Unknown	5 (1)	12 (1)	12 (1)
Other histologic type than ductal and lobular	56 (6)	53 (5)	145 (6)
Lymphovascular invasion			
Absent	856 (86)	859 (85)	1,925 (83)
Present	134 (13)	139 (14)	355 (15)
Unknown	11 (1)	13 (1)	28 (1)
ER status			
Negative (0%-9%)	299 (30)	273 (27)	680 (29)
Positive (≥ 10%)	702 (70)	738 (73)	1,628 (71)
HER2 status			
Positive (IHC 3+ or FISH ≥ 2.0)	113 (11)	109 (11)	329 (14)
Negative (IHC 0, 1+ and/or FISH < 2.0)	888 (89)	902 (89)	1,979 (86)
Ki-67			
Low (≤ 14%)	307 (31)	325 (32)	656 (28)
High (> 14%)	588 (59)	568 (56)	1,327 (57)
Unknown	106 (11)	118 (12)	325 (14)
Subtype			
Luminal A (ER positive, HER2 negative, and Ki-67 low)	277 (28)	284 (28)	584 (25)
Luminal B (ER positive and HER2 positive and/or Ki-67 high)	356 (36)	372 (37)	845 (37)
ER negative and HER2 normal	240 (24)	229 (23)	539 (23)
Unknown	128 (13)	126 (12)	340 (15)

Abbreviations: DC, docetaxel and cyclophosphamide; EC-D, epirubicin and cyclophosphamide followed by docetaxel; ER, estrogen receptor; FISH, fluorescent in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

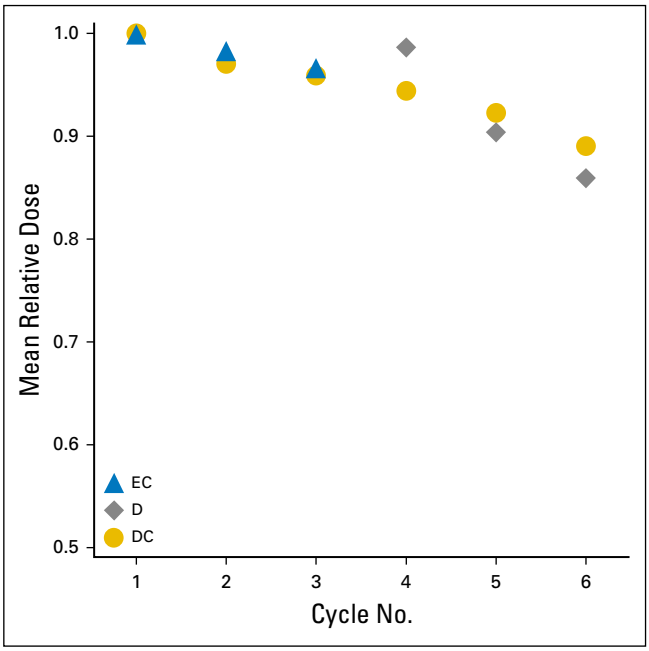

Fig 2. Mean relative dose per cycle (actual/planned mg/m^2). D, docetaxel; DC, docetaxel and cyclophosphamide; EC, epirubicin and cyclophosphamide.

cardiomyopathy after more than 6 years, whereas seven and six additional patients developed heart failure in the ED-C and DC groups, respectively. A second nonbreast primary invasive cancer was observed among 25 patients (2%) in the EC-D group and 27 patients (3%) in the DC group. Notably, two patients in the EC-D group developed acute myeloid leukemia (Table 2).

An exploratory ITT analysis of DFS (Fig 4A; Appendix Table A1) and OS (Fig 4B) in subgroups showed similar treatment effects by histologic subtype, nodal status, tumor size, ER expression, HER2 status, and Ki-67 expression but suggested differential effects by menopausal status and malignancy grade. Premenopausal patients had better DFS after DC, and postmenopausal patients had better DFS after EC-D (test of interaction, adjusted $P = .04$), and there was a suggestion of an effect in the same direction for OS (test of interaction, adjusted $P = .07$). Allocation to DC was associated with a significant benefit in patients with grade 3 tumors, whereas in patients with grade 1 to 2 tumors, allocation to EC-D was associated with improved DFS (test of interaction, adjusted $P = .02$) and OS (test of interaction, adjusted $P = .03$).

Toxicity

The overall proportion of patients reporting any acute grade 3 or 4 toxicity occurring during or within 21 days of last chemotherapy was significantly different in the two groups (Table 3). Grade 3 or 4 febrile neutropenia occurred more often ($P = .01$) in patients allocated to EC-D compared with patients allocated to DC.

The nonhematologic toxicity profile differed significantly (Table 3). Patients allocated to EC-D reported a significantly ($P < .001$) higher grade of stomatitis, myalgia or arthralgia, vomiting, nausea, and fatigue, whereas peripheral edema was more severe after DC. Peripheral neuropathy was significantly more common and severe ($P = .001$) with EC-D than with DC, whereas the rates of

Table 2. First Events According to the Intent-to-Treat Analysis

Event	No of Patients (%) EC-D (n = 1,001)	DC (n = 1,011)
All disease-free survival events	126 (13)	127 (13)
Local/regional invasive recurrence	17 (2)	10 (1)
Distant recurrence	61 (6)	65 (6)
Contralateral invasive breast cancer	10 (1)	5 (0)
Nonbreast second primary invasive cancer*	25 (2)	27 (3)
GI	9	3
Pancreas	1	2
Lung	4	4
Kidney	1	0
Gynecologic	3	10
Malignant melanoma	4	5
Sarcoma	0	1
Head and neck	1	0
Leukemia†	2	1
Non-Hodgkin lymphoma	0	1
Death, without recurrence	17 (2)	21 (2)
All deaths	68 (7)	78 (8)
Breast cancer related	42 (4)	47 (5)
Second nonbreast cancer	9 (1)	9 (1)
Not cancer related	17 (2)	22 (2)

Abbreviations: DC, docetaxel and cyclophosphamide; EC-D, epirubicin and cyclophosphamide followed by docetaxel.
*Encountering second nonbreast cancer events after a first.
†Two patients with acute myeloid leukemia in the EC-D group and one patient with chronic myeloid leukemia in the DC group.

skin disorders and nail changes were similar. Among 1,045 premenopausal women, 42 did not provide information about their menstrual periods, but among those who did, 8% in the EC-D group and 9% in the DC group reported menstruating regularly throughout chemotherapy, 12% in EC-D group and 10% in the DC group reported irregular menstruating, and 80% in the EC-D group and 81% in the DC group reported cessation of menses for 3 months or more after initiation of chemotherapy.

DISCUSSION

This analysis failed to demonstrate any overall outcome benefit from the anthracycline-containing adjuvant EC-D regimen compared with DC of similar duration in patients with *TOP2A*-normal tumors. Thus, our trial confirms the results hypothesized by a formal prospective-retrospective analysis of individual patient data from the DBCG 89D trial and four other trials.[9,14] Moreover, the sequential EC-D schedule was, apart from peripheral edema, associated with significantly more adverse events such as stomatitis, myalgia or arthralgia, peripheral neuropathy, vomiting, nausea, and fatigue. The high frequencies of nonhematologic adverse events may be a result of the use of patient-reported outcomes.[15,16] Despite a higher total dose and longer duration of docetaxel in the DC group, a significantly higher frequency of early peripheral neuropathy was observed in the EC-D group, which is likely to be explained by a higher dose (100 mg/m^2 in the EC-D group *v* 75 mg/ m^2 in DC group) per cycle. We previously have reported a significant reduction in the risk of peripheral neuropathy by use of frozen gloves and socks and a more pronounced late recovery in the EC-D group.[17] In both groups, docetaxel was accompanied by prophylactic colony-stimulating factor, and this may have been the reason for achieving a similar dose-intensity of epirubicin and cyclophosphamide and DC in the first three cycles. In contrast, febrile neutropenia was more pronounced during the following three cycles of docetaxel as compared with DC and may explain more extensive dose reductions. Nonetheless, the expectation of a change in adverse effects by the switch improved treatment adherence in the sequential treatment group.

Patients with grade 3 tumors had a favorable outcome from DC, whereas EC-D was more favorable in patients with grade 1 or 2 tumors, and this heterogeneity was significant in tests of interaction for DFS as well as OS. Histologic grade combines scores of tubule formation (glandular differentiation), nuclear pleomorphism, and

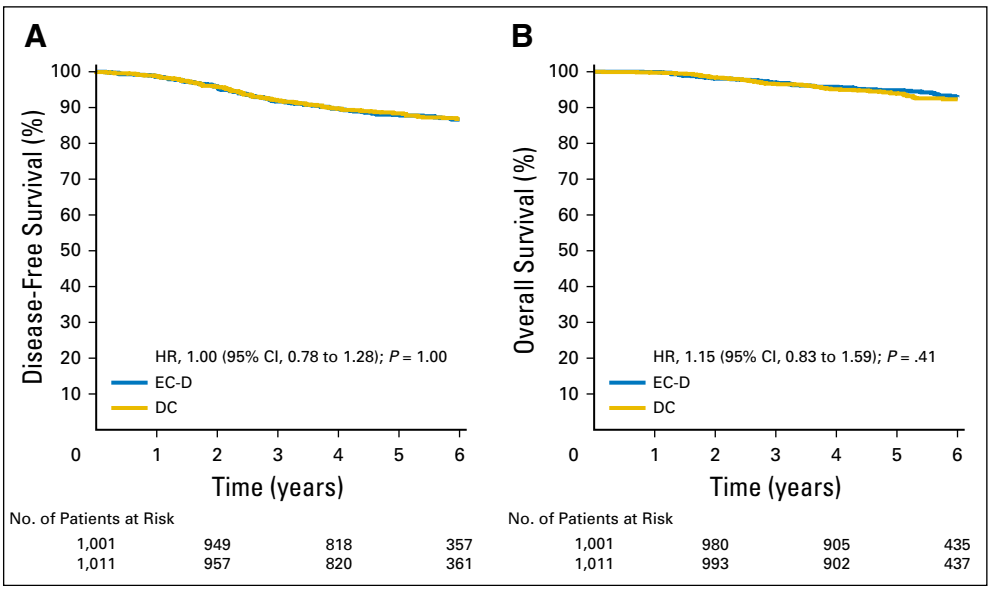

Fig 3. (A) Kaplan-Meier estimates of disease-free survival of the 2,012 patients included in the intent-to-treat analysis who were randomly allocated to epirubicin and cyclophosphamide followed by docetaxel (EC-D) or docetaxel and cyclophosphamide (DC). (B) Kaplan-Meier estimates of overall survival. HR, hazard ratio.

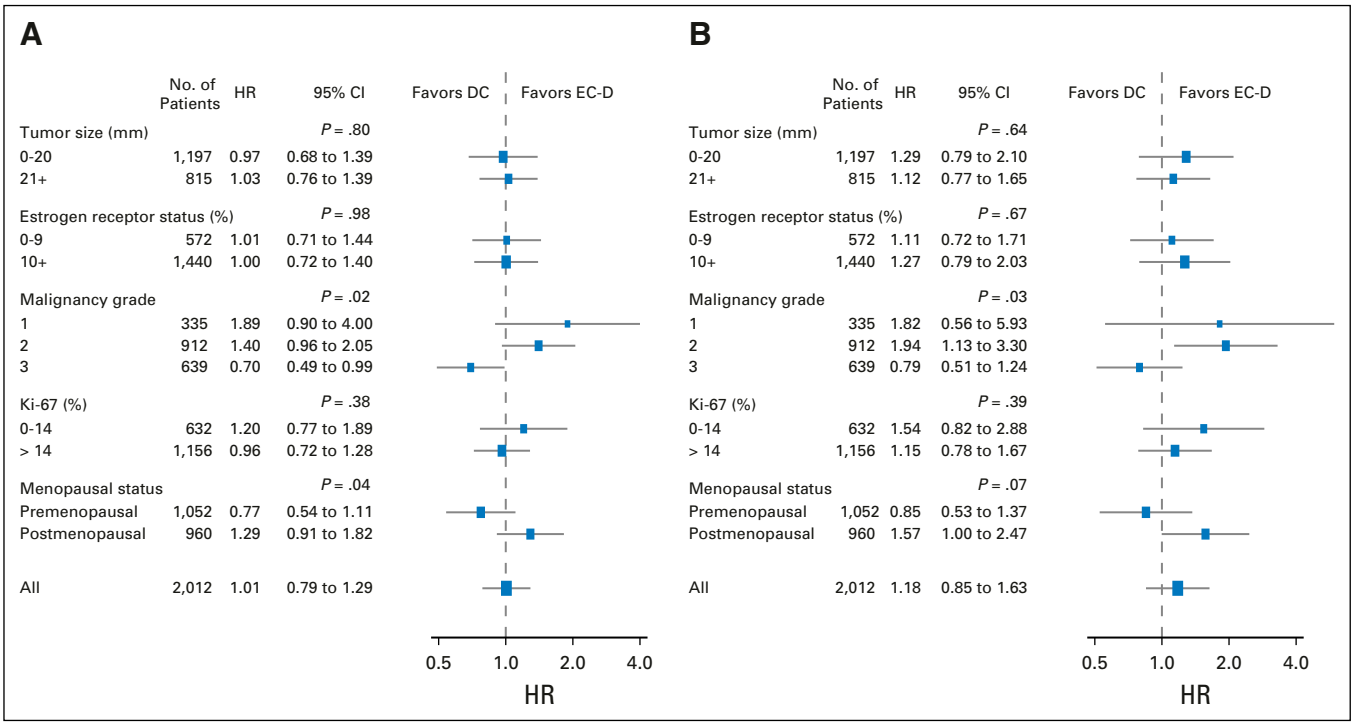

Fig 4. Forest plots illustrating proportional hazards models for (A) disease-free survival and (B) overall survival according to tumor size, estrogen receptor, malignancy grade, Ki-67, and menopausal status. Hazard ratios (HRs) refer to adjusted intent-to-treat estimates obtained in the multivariate analysis. *P* values are for test of heterogeneity of treatment effect. Boxes represent the weight of data for each subgroup relative to the total data. DC, docetaxel and cyclophosphamide; EC-D, epirubicin and cyclophosphamide followed by docetaxel.

mitotic counting and was standardized for its use in the Nottingham Prognostic Index by Elston and Ellis.[18,19] Grade and, in particular, mitotic count have been associated with proliferation as determined in multigene assays, and Ki-67 is also a measure of proliferation.[20] However, we found no evidence of an association between Ki-67 expression and a differential benefit from the two chemotherapy regimens. Ki-67 was not evaluated centrally, which may have been critical, and a predictive value of Ki-67 has not been demonstrated by others.[21-23] A high recurrence score was associated with benefit from adding CMF to tamoxifen in the National Surgical Adjuvant Breast and Bowel Project B-20 trial and from adding cyclophosphamide, doxorubicin, and fluorouracil to tamoxifen in Southwest Oncology Group trial 8814.[24,25] Similarly, a low-risk classifier (luminal A subtype) predicted lack of benefit from cyclophosphamide and CMF among premenopausal patients with node-positive breast cancer in DBCG 77B.[26] In the MA.5 trial, benefit from cyclophosphamide, epirubicin, and fluorouracil as compared with CMF was primarily confined to patients with an HER2-enriched subtype.[27]

In postmenopausal patients, EC-D was associated with an outcome benefit, whereas DC was favorable in premenopausal

Table 3. Frequency of Patient-Reported Adverse Events During Chemotherapy

	No. of Patients (%)										
	EC-D (n = 994)					DC (n = 1,006)					
Adverse Event	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	*P*
Hematologic											
Febrile neutropenia	800 (80)	60 (6)	15 (2)	87 (9)	9 (1)	855 (85)	66 (7)	13 (1)	49 (5)	10 (1)	.01
Nonhematologic											
Stomatitis	138 (14)	344 (35)	479 (48)	22 (2)	3 (0)	204 (20)	465 (46)	324 (32)	10 (1)	1 (0)	< .001
Diarrhea	362 (36)	391 (39)	166 (17)	60 (6)	7 (1)	324 (32)	431 (43)	185 (18)	53 (5)	11 (1)	.10
Myalgia/arthralgia	44 (4)	124 (12)	391 (39)	361 (36)	66 (7)	48 (5)	199 (20)	426 (42)	293 (29)	38 (4)	< .001
Peripheral neuropathy	264 (27)	373 (38)	228 (23)	97 (10)	24 (2)	321 (32)	386 (38)	212 (21)	57 (6)	28 (3)	.001
Skin disorder	356 (36)	447 (45)	158 (16)	25 (3)	—	379 (38)	457 (45)	154 (15)	14 (1)	—	.26
Nail disorder	270 (27)	577 (58)	139 (14)	—	—	282 (28)	600 (60)	122 (12)	—	—	.37
Vomiting	596 (60)	190 (19)	141 (14)	52 (5)	7 (1)	783 (78)	149 (15)	65 (6)	1 (0)	6 (1)	< .001
Nausea	103 (10)	465 (47)	340 (34)	71 (7)	7 (1)	255 (25)	552 (55)	182 (18)	11 (1)	4 (0)	< .001
Fatigue	8 (1)	255 (26)	427 (43)	249 (25)	48 (5)	33 (3)	290 (29)	436 (43)	225 (22)	20 (2)	< .001
Peripheral edema	387 (39)	464 (47)	110 (11)	25 (3)	—	334 (33)	463 (46)	181 (18)	26 (3)	—	< .001

Abbreviations: DC, docetaxel and cyclophosphamide; EC-D, epirubicin and cyclophosphamide followed by docetaxel.

patients. The test of interaction between menopausal status and treatment group was statistically significant for DFS but not for OS. Total cyclophosphamide dose received and the use of anthracyclines have been emphasized as most important concerning chemotherapy-induced menopause, whereas taxanes are of less importance.[8,28,29] Amenorrhea was equally frequent after DC and EC-D, but in the absence of biochemical monitoring of ovarian function, an endocrine effect may not be completely excluded. Adherence to antiestrogens was similar in the two groups.

Several issues should be considered when interpreting the 5-year results from this trial. First, our trial was designed as a superiority trial. Although the trial met its recruitment goal, the number of events was lower than expected, as was the resulting statistical power. Second, in the design, emphasis was given to the selection of internationally accepted docetaxel regimens and to using chemotherapy of the same duration, and accordingly, a duration of six cycles was selected to be used in both groups. Third, mechanisms of action unrelated to topoisomerase IIα have been proposed for anthracyclines including genetic instability reflected by polysomy of centromere 17 (CEN17) and protection from apoptosis by tissue inhibitor of matrix metalloproteinases-1 (TIMP-1).[30,31] Finally, central testing by a validated, US Food and Drug Administration–approved, and CE labeled *TOP2A* test was applied in the 07-READ trial. These results may not be applicable using other *TOP2A* tests because the quality control preformed within the international individual-patient pooled analysis of HER2 and *TOP2A* revealed a suboptimal reproducibility between different *TOP2A* platforms and institutions.[14]

In conclusion, EC-D did not demonstrate any overall outcome advantage compared with DC in patients with a *TOP2A*-normal tumor, and EC-D had a less favorable toxicity profile. Although the data are in favor of DC in patients with *TOP2A*-normal breast cancer, a statistical heterogeneity was identified, and a subgroup of patients may therefore derive benefit from treatment with anthracyclines.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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REFERENCES

1. Early Breast Cancer Trialists' Collaborative Group (EBCTCG): Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: An overview of the randomised trials. Lancet 365:1687-1717, 2005
2. Peto R, Davies C, Godwin J, et al: Comparisons between different polychemotherapy regimens for early breast cancer: Meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet 379:432-444, 2012
3. Paik S, Bryant J, Tan-Chiu E, et al: HER2 and choice of adjuvant chemotherapy for invasive breast cancer: National Surgical Adjuvant Breast and Bowel Project Protocol B-15. J Natl Cancer Inst 92: 1991-1998, 2000
4. Pritchard KI, Shepherd LE, O'Malley FP, et al: HER2 and responsiveness of breast cancer to adjuvant chemotherapy. N Engl J Med 354:2103-2111, 2006
5. Blum JL, Flynn PJ, Yothers G, et al: Interim joint analysis of the ABC (anthracyclines in early breast cancer) phase III trials (USOR 06-090, NSABP B-46I/USOR 07132, NSABP B-49 [NRG Oncology]) comparing docetaxel + cyclophosphamide (TC) v anthracycline/taxane-based chemotherapy regimens (TaxAC) in women with high-risk, HER2-negative breast cancer. J Clin Oncol 34, 2016 (suppl; abstr 1000)
6. Baxter J, Sen N, Martínez VL, et al: Positive supercoiling of mitotic DNA drives decatenation by topoisomerase II in eukaryotes. Science 331: 1328-1332, 2011
7. Kellner U, Sehested M, Jensen PB, et al: Culprit and victim: DNA topoisomerase II. Lancet Oncol 3:235-243, 2002
8. Ejlertsen B, Mouridsen HT, Jensen MB, et al: Improved outcome from substituting methotrexate with epirubicin: Results from a randomised comparison of CMF versus CEF in patients with primary breast cancer. Eur J Cancer 43:877-884, 2007
9. Knoop AS, Knudsen H, Balslev E, et al: Retrospective analysis of topoisomerase IIa amplifications and deletions as predictive markers in primary breast cancer patients randomly assigned to cyclophosphamide, methotrexate, and fluorouracil or cyclophosphamide, epirubicin, and fluorouracil: Danish Breast Cancer Cooperative Group. J Clin Oncol 23: 7483-7490, 2005
10. Christiansen P, Ejlertsen B, Jensen MB, et al: Danish Breast Cancer Cooperative Group. Clin Epidemiol 8:445-449, 2016
11. Olsen KE, Knudsen H, Rasmussen BB, et al: Amplification of HER2 and TOP2A and deletion of TOP2A genes in breast cancer investigated by new FISH probes. Acta Oncol 43:35-42, 2004
12. Henriksen KL, Rasmussen BB, Lykkesfeldt AE, et al: Semi-quantitative scoring of potentially predictive markers for endocrine treatment of breast cancer: A comparison between whole sections and tissue microarrays. J Clin Pathol 60:397-404, 2007
13. Royston P, Sauerbrei W: A new approach to modelling interactions between treatment and continuous covariates in clinical trials by using fractional polynomials. Stat Med 23:2509-2525, 2004
14. Di Leo A, Desmedt C, Bartlett JM, et al: HER2 and TOP2A as predictive markers for anthracycline-containing chemotherapy regimens as adjuvant treatment of breast cancer: A meta-analysis of individual patient data. Lancet Oncol 12:1134-1142, 2011
15. Ellis P, Barrett-Lee P, Johnson L, et al: Sequential docetaxel as adjuvant chemotherapy for early breast cancer (TACT): An open-label, phase III, randomised controlled trial. Lancet 373:1681-1692, 2009
16. Basch E, Iasonos A, McDonough T, et al: Patient versus clinician symptom reporting using the National Cancer Institute Common Terminology Criteria for Adverse Events: Results of a questionnaire-based study. Lancet Oncol 7:903-909, 2006
17. Eckhoff L, Knoop AS, Jensen MB, et al: Risk of docetaxel-induced peripheral neuropathy among 1,725 Danish patients with early stage breast cancer. Breast Cancer Res Treat 142:109-118, 2013
18. Haybittle JL, Blamey RW, Elston CW, et al: A prognostic index in primary breast cancer. Br J Cancer 45:361-366, 1982
19. Balslev I, Axelsson CK, Zedeler K, et al: The Nottingham Prognostic Index applied to 9,149 patients from the studies of the Danish Breast Cancer Cooperative Group (DBCG). Breast Cancer Res Treat 32:281-290, 1994
20. Rakha EA, Reis-Filho JS, Baehner F, et al: Breast cancer prognostic classification in the molecular era: The role of histological grade. Breast Cancer Res 12:207, 2010
21. Polley MY, Leung SC, Gao D, et al: An international study to increase concordance in Ki67 scoring. Mod Pathol 28:778-786, 2015

22. Ejlertsen B, Jensen MB, Elversang J, et al: One year of adjuvant tamoxifen compared with chemotherapy and tamoxifen in postmenopausal patients with stage II breast cancer. Eur J Cancer 49:2986-2994, 2013

23. Viale G, Regan MM, Mastropasqua MG, et al: Predictive value of tumor Ki-67 expression in two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer. J Natl Cancer Inst 100:207-212, 2008

24. Paik S, Tang G, Shak S, et al: Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. J Clin Oncol 24:3726-3734, 2006

25. Albain KS, Barlow WE, Shak S, et al: Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: A retrospective analysis of a randomised trial. Lancet Oncol 11:55-65, 2010

26. Nielsen TO, Jensen MB, Burugu S, et al: High risk premenopausal luminal A breast cancer patients derive no benefit from adjuvant cyclophosphamide-based chemotherapy: Results from the DBCG77B clinical trial. Clin Cancer Res 23:946-953, 2017

27. Cheang MC, Voduc KD, Tu D, et al: Responsiveness of intrinsic subtypes to adjuvant anthracycline substitution in the NCIC.CTG MA.5 randomized trial. Clin Cancer Res 18:2402-2412, 2012

28. Ejlertsen B, Mouridsen HT, Jensen MB, et al: Cyclophosphamide, methotrexate, and fluorouracil; oral cyclophosphamide; levamisole; or no adjuvant therapy for patients with high-risk, premenopausal breast cancer. Cancer 116:2081-2089, 2010

29. Pérez-Fidalgo JA, Roselló S, García-Garré E, et al: Incidence of chemotherapy-induced amenorrhea in hormone-sensitive breast cancer patients: The impact of addition of taxanes to anthracycline-based regimens. Breast Cancer Res Treat 120: 245-251, 2010

30. Bartlett JM, McConkey CC, Munro AF, et al: Predicting anthracycline benefit: TOP2A and CEP17—Not only but also. J Clin Oncol 33:1680-1687, 2015

31. Ejlertsen B, Jensen MB, Nielsen KV, et al: HER2, TOP2A, and TIMP-1 and responsiveness to adjuvant anthracycline-containing chemotherapy in high-risk breast cancer patients. J Clin Oncol 28: 984-990, 2010

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Adjuvant Cyclophosphamide and Docetaxel With or Without Epirubicin for Early *TOP2A*-Normal Breast Cancer: DBCG 07-READ, an Open-Label, Phase III, Randomized Trial

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Appendix

Table A1. Cox Proportional Hazards Model for DFS

Parameter	HR	95% CI	*P*
Age at enrollment: continuous	1.01	0.99 to 1.02	.25
Tumor size: log(tumor size [mm]/100)	1.89	1.45 to 2.46	< .001
Nodal status: log([No. of positive nodes + 1]/10)	1.45	1.24 to 1.71	< .001
ER status, %: continuous	0.992	0.988 to 0.996	< .001
Lymphovascular invasion: present	1.74	1.26 to 2.39	< .001
HER2 status: positive	1.15	0.80 to 1.67	.45
Ki-67, %: continuous	1.003	0.997 to 1.009	.34
Malignancy grade: ordered	1.29	1.00 to 1.67	.05
Treatment regimen: DC *v* EC-D	1.01	0.79 to 1.29	.96

Abbreviations: DC, docetaxel and cyclophosphamide; DFS, disease-free survival; EC-D, epirubicin and cyclophosphamide followed by docetaxel; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hazard ratio.