

Cyclooxygenase-2 Inhibition Does Not Improve the Reduction in Ductal Carcinoma *In situ* Proliferation with Aromatase Inhibitor Therapy: Results of the ERISAC Randomized Placebo-Controlled Trial

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Abstract

Purpose: Tamoxifen reduces risk of recurrence after breast conservation surgery for ductal carcinoma *in situ* (DCIS), but no data exists on the effectiveness of aromatase inhibitors for DCIS. Cyclooxygenase-2 (COX-2) is overexpressed in DCIS, representing another potential therapeutic target. The aim of the study was to determine the effect of aromatase and/or COX-2 inhibition on epithelial proliferation and apoptosis in a presurgical study of estrogen receptor (ER)-positive DCIS.

Methods: Postmenopausal women with ER-positive DCIS diagnosed by core biopsy were randomized to a 2 × 2 design of either 14 days of exemestane or placebo and celecoxib, or placebo immediately before surgery. Paired baseline and end point biopsies were analyzed for proliferation (Ki67), apoptosis, human epidermal growth factor receptor 2 (HER2), COX-2, and progesterone receptor (PR) expression by immunohistochemistry. The primary end point was a decrease in Ki67 between diagnosis and surgical excision.

Results: Ninety women were randomized: all were ER positive, 49 (54%) had grade III tumors, and 29 (32%) were HER2 positive (3+). Exemestane reduced proliferation compared with placebo with a median reduction of 9% (95% confidence interval, 6-14; $P < 0.001$). Progesterone receptor was reduced by exemestane (mean decrease, 19%; 95% confidence interval, 9-28; $P = 0.011$). The effect of exemestane on proliferation was seen regardless of grade, HER2, or PR expression. Celecoxib had no effect on proliferation or apoptosis alone, or in combination with exemestane.

Conclusions: Exemestane reduces proliferation in ER-positive DCIS. Aromatase inhibition is a potential alternative to tamoxifen in patients who have undergone breast conservation for ER-positive DCIS. *Clin Cancer Res*; 16(5); 1605-12. ©2010 AACR.

Ductal carcinoma *in situ* (DCIS) accounts for 25% of all the screen-detected breast cancer (1, 2). Most patients with DCIS are treated with breast conservation surgery with subsequent adjuvant therapy with radiation and/or tamoxifen (1, 2). However, conservation surgery is associated with local recurrence rates of between 7% and 30% after 10 years of follow-up compared with <1% following

mastectomy (3). Approximately half of all recurrences are invasive cancers (1, 2).

Studies investigating the benefit of adjuvant tamoxifen in DCIS have shown either limited or no benefit for this treatment (1, 2). The NSAPB B24 Trial treated all patients after wide local excision with radiotherapy and randomized them to either tamoxifen or placebo. A 50% relative risk reduction in all breast cancer events in patients treated with tamoxifen primarily by reduction in the frequency of ipsilateral invasive cancer was found (1). Subsequent retrospective reanalysis of these data by Allred (4) suggests that the benefit of tamoxifen was confined to those who had estrogen receptor (ER)-positive DCIS with no effect being seen in the ER-negative DCIS. In the United Kingdom/ANZ DCIS study, tamoxifen had little effect in preventing recurrence (2).

DCIS expresses human epidermal growth factor receptor 2 (HER2) in up to 60% of high-grade lesions and expresses epidermal growth factor receptor (ErbB) in the majority (70%) of lesions (5, 6). Experimental and clinical

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Translational Relevance

Tamoxifen is known to reduce recurrence after breast conservation surgery for ductal carcinoma *in situ* (DCIS) but its effectiveness in human epidermal growth factor receptor 2 (HER2)-positive DCIS is unknown. Although animal models and randomized trials have shown biological effects by targeting the estrogen receptor (ER) in DCIS, this is the first prospective randomized trial in ER-positive DCIS that confirms that targeting the ER inhibits proliferation and increases apoptosis. Inhibition of DCIS epithelial proliferation by exemestane in a placebo-controlled trial provides proof of principle that aromatase inhibitors will do likewise in the current ongoing adjuvant trials in DCIS. Effects of aromatase inhibition were seen in HER2-positive and HER2-negative DCIS. Cyclooxygenase-2 is expressed in DCIS but the cyclooxygenase-2 inhibitor celecoxib had no effect on either proliferation or apoptosis and is unlikely to have therapeutic value in DCIS.

studies indicate that invasive tumors that express either HER2 or epidermal growth factor receptor respond more to estrogen reduction by aromatase inhibition than ER blockade by tamoxifen. MCF-7 cells (an ER-positive breast cancer cell line) transfected with the HER2 neu gene were tamoxifen resistant but responded to estrogen (7), and clinical studies indicate a greater response to aromatase inhibitors in patients with ER-positive HER2-positive tumors (8). These data suggest that HER2-positive DCIS may respond better to aromatase inhibitors (5).

The aim was to test whether ER-positive DCIS responds to aromatase inhibition (compared with placebo) by measuring epithelial proliferation and apoptosis.

Cyclooxygenase-2 (COX-2) is an enzyme that is overexpressed in 70% of DCIS compared with normal breast epithelium (5, 9). COX-2 expression is increased in HER2-positive tumors and is associated with increased expression of the aromatase enzyme. COX-2 increases aromatase activity and inhibitors of COX-2 such as celecoxib reduce aromatase activity (10) and increase apoptosis leading to tumor growth inhibition in animal models (11). Despite claims that the use of COX-2 inhibition may potentially prevent progression of DCIS to invasive disease, little evidence exists in human DCIS to support this hypothesis (12, 13). A further aim of this study was to test whether a COX-2 inhibitor (celecoxib) decreases proliferation or increases apoptosis after 14 days of treatment either when used alone or in combination with exemestane. Because COX-2 is also expressed in blood vessels around tumors and seems to exert antiangiogenic activity in animal models, a further secondary aim was to assess the effect of COX-2 inhibition on serum markers of angiogenesis.

Patients, Materials, and Methods

Postmenopausal women (defined as age 50 y and/or no periods for >2 mo or having follicle-stimulating hormone/luteinizing hormone of >25 IU/L in hysterectomized patients) with a core biopsy diagnosis of ER-positive DCIS or DCIS and invasive cancer presenting to breast clinics were screened for and gave written informed consent to allow entry to the trial. Patients were randomized at four centers within the United Kingdom and underwent definitive surgical excision of the DCIS within 30 d (range, 15-57) of core biopsy (Fig. 1). Patients ($n = 401$) with ER-negative DCIS ($n = 121$) on core biopsy or who did not give informed consent ($n = 62$) were excluded from the study, as were patients who had received primary chemotherapy (neoadjuvant) treatment or who had used nonsteroidal antiinflammatory drugs, hormone replacement therapy (HRT), or aromatase inhibitors in the 3 mo before diagnosis ($n = 218$). Patients were randomly assigned to receive either oral 25 mg/d exemestane or identical placebo exemestane tablets and were also randomized in a 2×2 manner either to 800 mg/d celecoxib orally or equivalent celecoxib placebo tablets (Fig. 2A). Randomization was done by the University Hospital of South Manchester NHS Foundation Trust Department of Medical Statistics. Pfizer Pharmaceuticals packaged the study drug materials but was not involved in the study design or patient monitoring. All patients took study drugs following core biopsy for the 14 d immediately before surgical resection of the primary tumor. Tumor grade and size were measured by local breast pathologists using standardized National Health Service Breast Screening Program pathology reporting guidelines.

Immunohistochemical assays for Ki67, ER, progesterone receptor, COX-2, and HER2. Paraffin-embedded blocks from all patients were sent to the Breast Biology Laboratory at the Paterson Institute for Cancer Research, Manchester. Paired paraffin wax sections of 2 to 5 μm of tissue from the diagnostic core biopsy and surgical DCIS excision specimens of each patient were mounted on 3-aminopropyltriethoxysilane and Sigma-coated slides, dewaxed in xylene, and rehydrated before immunohistochemical staining for Ki67 nuclear antigen labeling (a marker of cellular proliferation), ER, progesterone receptor (PR), and HER2 on DCIS from both sample pairs. Ki67 was detected using the MIB-1 monoclonal antibody in the DCIS component. Methods for Ki67, ER, PR, and HER2 have been previously described in ref. (10). Apoptosis was measured using terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling deoxynucleotide labeling using a commercial kit. COX-2 staining was measured as previously described in ref. (5). A primary goat polyclonal anti-human COX-2 antibody was used (Santa Cruz Biotechnology) and COX-2 cytoplasmic staining was scored as 0 (absent), 1+ (weak), 2+ (moderate), and 3+ (strong) based on the extent and intensity of epithelial cell staining (5). In every case, at least 1,000 cells were counted for scoring. For molecular markers, antigen retrieval was

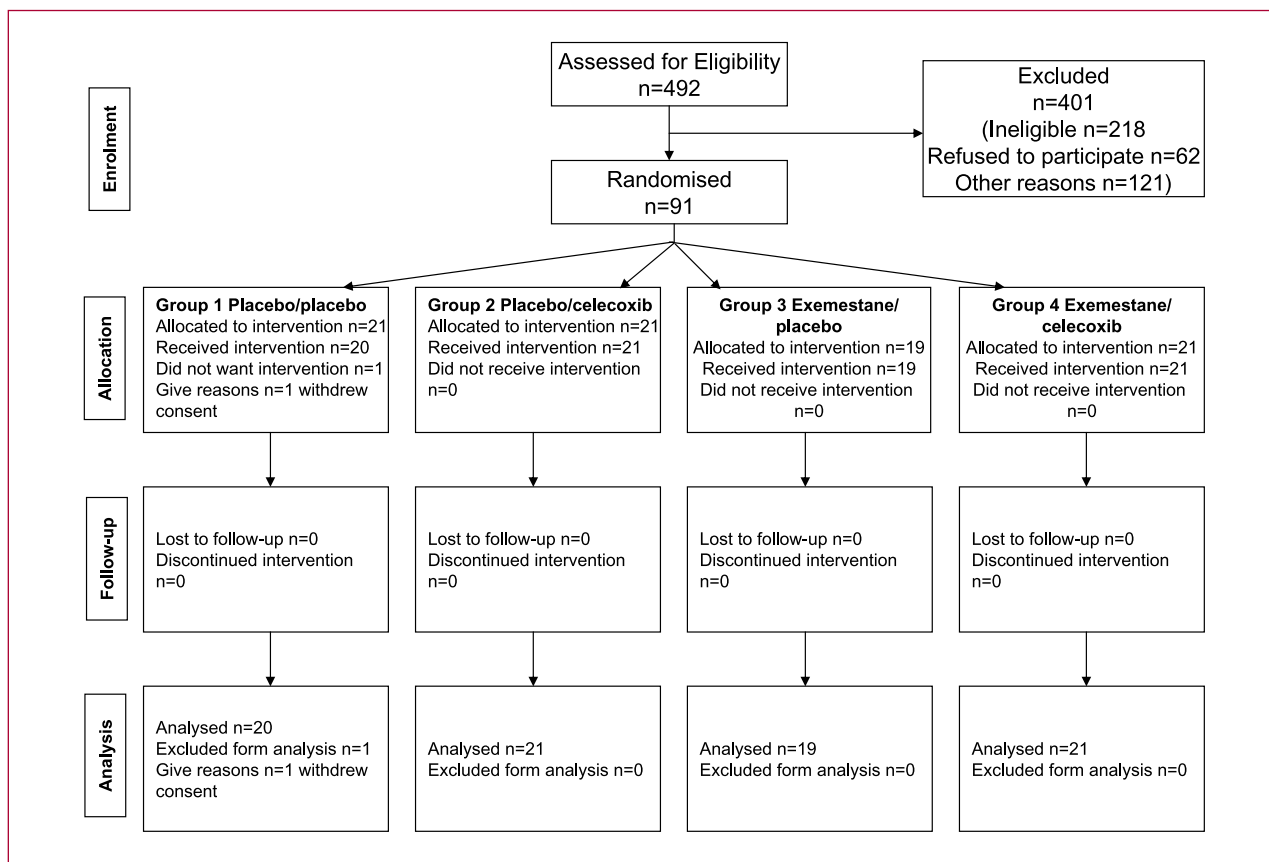


Fig. 1. CONSORT diagram.

achieved by the pressure cooking method for 4 min in citrate buffer (pH 6.0). Previously described methods were used (5, 11).

Slides were reviewed by a pathologist in Manchester (Prof. G Landberg) to ensure that immunohistochemistry markers were done on DCIS.

Immunohistochemical staining was nuclear for Ki67, ER, and PR, and predominant in the cell membrane for HER2 (5). For COX-2, a minimum of 500 cells was sought across randomly selected areas of DCIS at a magnification of $\times 400$ using a grid graticule and cell counter for each of the two sections. Ki67, ER, and PR scores were calculated as a percentage of positively stained nuclei (i.e., positive cells/total number of cells $\times 100\%$). ER and PR positivity was defined as $>5\%$ stained nuclei consistent with that used for invasive breast cancer. HER2 staining was scored as 0 (absent) to 3 (maximum cytomembranous staining seen, comparable with invasive cancer control), with a score of >2 considered HER2 positive. Scoring was carried out by two investigators who were blinded to treatment assignment; if scores disagreed, differences were resolved by agreement after a double-headed microscopic examination.

Serum angiogenic markers. Serum was collected at baseline and on the day of surgery from all women who entered the trial; the serum was stored at -80°C . Subsequently, paired serum samples from each patient

were analyzed for VCAM and vascular endothelial growth factor (VEGF) using commercial ELISA assays according to the manufacturer's instructions (R and D).

Statistical analyses. Primary end point was an absolute reduction in DCIS epithelial proliferation as measured by the Ki67 labeling index. To calculate the sample size, we used a previous study in our center of women on HRT diagnosed with DCIS who stopped treatment after core biopsy and underwent surgery 14 d later (14), leading to a decrease in proliferation (Ki67) of 30% in women stopping HRT. We expected $\sim 70\%$ of women with exemestane to have an absolute drop in proliferation of at least 50% compared with none of the placebo group (equates to a probability of 0.71 for a drop in proliferation greater in the exemestane group, using a Mann-Whitney *U* test). Thus, this study had a 90% power with 80 women overall for the exemestane versus placebo comparison. The primary end point of Ki67 was calculated to require a minimum of 90 evaluable patients with the assumption that 10% of patients enrolled would not be evaluable. In the event, 10 patients were nonevaluable due to the lack of paired or accessible samples (9 patients) and 1 patient withdrew before commencing 14 d of treatment. Comparison was made against the placebo arm on an intention-to-treat basis with respect to changes in biomarker measurements between pretreatment and

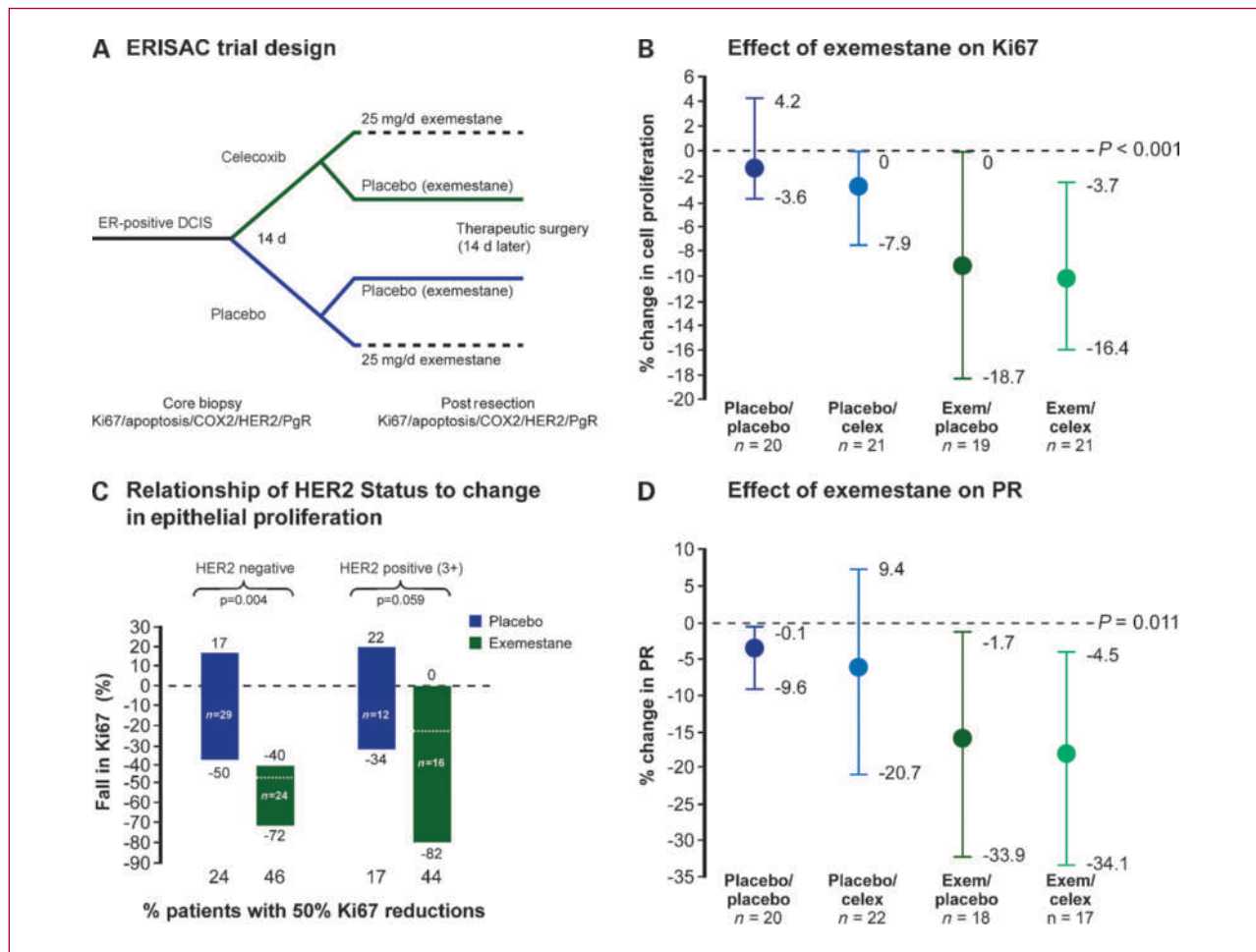


Fig. 2. Trial design and results. A, ERISAC trial design showing 2 × 2 randomization. B, proliferation Ki67 decrease from baseline in exemestane-treated but not placebo-treated patients in ER-positive DCIS was 9% (95% CI, 6-14; $P \leq 0.001$). C, HER2-negative tumors had a significant decrease in proliferation. D, exemestane significantly reduced PR in ER-positive DCIS decrease from baseline of 19% (95% CI, 9-28; $P = 0.011$).

posttreatment samples. The Ki67 data were not normally distributed and thus the Mann-Whitney U test was used to test the main effects in the factorial design by combining subgroups as convention. Analyses of covariance, taking appropriate account of the factorial design, were used to assess apoptosis and PR changes. The strength of the relationships between cell proliferation and other variables was measured using Spearman correlations. All of the safety analyses included data from randomized patients.

Results

Randomization was split evenly across the four groups (Table 1) of placebo and placebo, celecoxib and placebo, exemestane and placebo, and exemestane and celecoxib. One patient withdrew from the study in the placebo/placebo group (Table 2). The median DCIS size was 21 mm (range, 1-100 mm) and 99%, 72%, 32%, and 58% of DCIS were ER, PR, HER2, and COX-2 positive, respectively. There were no differences in distribution of receptor

status between the four study groups (Table 1). More than 98% of patients took the study drug for 13 days or longer.

Proliferation. DCIS from patients randomized to exemestane had a median 9% absolute reduction in cell proliferation [95% confidence interval (95% CI), 6 to 14; $P < 0.001$], which represented a median relative percentage decrease of 50% (95% CI, 34-71; $P < 0.001$) compared with placebo at 0% (95% CI, -16 to 26). No effects of celecoxib on proliferation was seen with a median absolute reduction of 7% (95% CI, 3-9; $P = 0.26$) compared with placebo (2%; 95% CI, -2 to 9; Table 3). Exemestane and celecoxib produced a 10% absolute reduction in proliferation (95% CI, 4 to -16) compared with a 9% (95% CI, 0-19) reduction with exemestane alone ($P = 0.71$).

The decrease in Ki67 on exemestane occurred regardless of the presence or absence of coexistent invasive cancer. Forty-five percent of exemestane-treated patients had a 50% drop, whereas only 20% of placebo-treated patients had changes in proliferation of this magnitude (Table 2).

We investigated the relationship of HER2 status, tumor grade, and receptor status to the change in

Table 1. Baseline characteristics of all randomized patients

	Placebo/placebo (n = 22)	Celex/placebo (n = 23)	Exem/placebo (n = 22)	Exem/celex (n = 23)
Median age (range)	62 (54-75)	60 (53-78)	62 (53-79)	64 (50-84)
Grade I	14% (3)	13% (3)	5% (1)	0% (0)
II	27% (6)	30% (7)	36% (8)	52% (12)
III	59% (13)	52% (12)	55% (12)	48% (11)
Invasive cancer	52% (12)	44% (10)	50% (11)	35% (8)
Median tumor size (range)	15 (5-100)	23 (9-60)	27 (1-68)	17 (1-80)
PR positive	68% (15)	78% (18)	71% (15)	68% (15)
HER2 positive (3+)	32% (7)	26% (6)	36% (8)	35% (8)
COX-2 positive (3+)	62% (13)	59% (13)	45% (9)	64% (14)

NOTE: All patients took 14 d of study drugs in the 14 d immediately preceding surgery.

Abbreviation: Exem, exemestane.

epithelial proliferation on exemestane. For HER2-negative DCIS, a median decrease in proliferation of 50% (95% CI, 40-72; $P = 0.004$) was seen on exemestane compared with placebo (median, 0%; 95% CI, -17 to 38). For HER2-positive (3+) tumors, however, the decrease was 35% (95% CI, 0-82; $P = 0.059$) on exemestane compared with placebo (-1%; 95% CI, -22 to 34). For those tumors that were PR positive, there was a significant (50%) decrease in proliferation (95% CI, 27-76) compared with placebo (19% decrease; 95% CI, -9 to 38; $P = 0.006$), whereas those that were PR negative had a lower percentage decrease in Ki67 of 39% (95% CI, -6 to 69; $P = 0.03$) compared with placebo (an increase of 17%; 95% CI, -9 to 155). Tumor grade did not affect overall response to exemestane. Among grades 1+2 DCIS, 9 of 19 (47%) patients on exemestane had a >50% decrease compared with 2 of 18 (11%) on placebo. For grade 3 DCIS, 8 of 20 (40%) patients had a >50% decrease on exemestane compared with 6 of 22 (27%) on placebo.

Apoptosis. Neither exemestane nor celecoxib affected apoptosis (12% increase, $P = 0.99$ and 15% increase, $P = 0.82$, respectively), but there was a small increase in apoptosis that occurred in celecoxib/placebo and exemestane/

placebo groups, which was not seen in the exemestane/celecoxib group (11% decrease), although the extent of this interaction did not reach significance ($P = 0.08$).

In the exemestane-treated patients, a significant decrease in Ki67 for an individual tumor was associated with an increase in apoptosis in the same tumor (Spearman ρ correlation for % change = -0.37; $P = 0.03$); no such correlation was seen in the placebo arm (Spearman ρ correlation for % change = 0.16; $P = 0.34$). The increase in apoptosis occurred predominantly in HER2-negative rather than HER2-positive DCIS treated with exemestane.

Effect of treatment on PR. PR expression was inversely associated with HER2 expression. Only 46% of HER2-positive DCIS were PR positive compared with 83% of HER2-negative DCIS ($P = 0.001$). Exemestane produced a decrease in expression of PR amounting to an absolute decrease of 19% (95% CI, 9-28; $P = 0.011$) compared with placebo. No effect of celecoxib on PR was seen ($P = 0.61$). The decrease in PR with exemestane was seen in HER2-negative DCIS (-19.9%; 95% CI, -9 to 38; $P = 0.027$) but not in HER2-positive DCIS (-8.0% exemestane versus -4.3% on placebo; $P = 0.65$). No additional effect of combining exemestane and celecoxib was seen on PR expression.

Table 2. Side effects seen in randomized groups

	Placebo/Placebo	Celex/Placebo	Exem/Placebo	Exem/Celex
Paired samples available	20	21	19	21
Unpaired/nonassessable	1	2	3	2
Withdrawals	1	0	0	0
Adverse events (%)	43%	39%	36%	52%
None	10%	0%	23%	9%
Headache	12%	17%	4%	13%
Hot flushes	10%	17%	9%	4%
Nausea				

Abbreviation: Celex, celecoxib.

COX-2 expression and marker response. COX-2 positivity (previously defined as 2+ or greater staining; ref. 10) was seen in 69 of 90 (77%) DCIS: 17 of 69 (25%) were HER2 positive, whereas 10 of 16 (62%; $P = 0.008$) COX-2-negative DCIS were HER2 positive. There was no change in apoptosis or proliferation in response to celecoxib in COX-2+ve compared with COX-2-negative DCIS. No change in COX-2 expression was seen in those patients treated with celecoxib and COX-2 expression was unrelated to grade or PR expression.

Serum angiogenic assays. Serum levels of VCAM and VEGF at baseline were similar in the four groups and did not change significantly with treatment (Table 3).

Adverse events. In patients treated with exemestane and celecoxib, the major side effects were headaches (7 of 68, 10%), hot flushes (8 of 68, 12%), and nausea (7/68, 10%). Similar side effects were seen in women taking placebo [headaches (2 of 22, 9%), hot flushes (1 of 22, 4%), and nausea (2 of 22; 9%); Table 2].

Discussion

Although animal models and randomized trials (13, 14) have shown biological effects by targeting the ER in DCIS, this is the first prospective randomized trial in ER-positive DCIS that confirms targeting the ER inhibits proliferation and increases apoptosis. Treatment with exemestane resulted in a decrease in proliferation of ~50%

(similar to previous reports in invasive tumors); however, aromatase inhibitors are associated with a decrease in the rate of apoptosis in invasive tumors, whereas we found an increase in apoptosis in tumor cells within DCIS. The reason for this difference between noninvasive and invasive tumors requires further investigation (12). The decrease in PR expression was similar to that reported in invasive tumors (12). No effect on biological end points was seen with celecoxib alone or in combination with exemestane.

An uncontrolled study on the effects of aromatase inhibitors on DCIS was reported by Dixon et al. (13) who showed decreases in proliferation in over 90% of patients treated with the nonsteroidal aromatase inhibitors, letrozole, and anastrozole who had DCIS on core biopsy adjacent to invasive cancers. The use of placebo in preoperative studies is essential because changes in proliferation could potentially be produced by inflammation as a result of the initial core biopsy.

In patients with invasive tumors, the short-term decrease in tumor cell proliferation in response to estrogen deprivation with either an aromatase inhibitor or tamoxifen is associated with an improvement in disease-free and overall survival. Thus, Ki67 estimation seems to be an intermediate end point of the efficacy of endocrine treatment for invasive disease but it remains to be determined whether such a decrease is associated with reduced local relapse of DCIS. The 50% median decrease in proliferation was

Table 3. Trial results

	Group 1		Group 2		Group 3		Group 4		Significance*
	Placebo/ placebo (n = 20)		Placebo/ celecoxib (n = 21)		Exemestane/ placebo (n = 19)		Exemestane/ celecoxib (n = 21)		
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
Ki67% Median (range)	17.2 (3.1-27.0)	13.5 (2.7-28.0)	13.5 (3.2-50.0)	11.6 (1.8,44.6)	20.7 (0.2-52.0)	10.1 (.05-52.0)	19.8 (6.9-49.2)	9.3 (0.6-36.6)	E: $P \leq 0.001$; C: $P = 0.26$
Apoptosis %, mean (range)	1.81 (0.33-6.40)	1.81 (0.81-4.70)	2.01 (0.38-7.30)	2.32 (0.96-7.54)	2.02 (0.54-6.50)	2.27 (0.92-4.90)	2.3 (0.47-9.40)	2.05 (0.78-6.10)	E: $P = 0.99$; C: $P = 0.82$; ExC: $P = 0.08$
PR %, mean (range)	39.7 (0-100)	34.9 (0-100)	50.7 (0-100)	45 (0-100)	36.6 (0-100)	18.8 (0-90)	47.4 (0-100)	28.1 (0-100)	E: $P = 0.011$; C: $P = 0.61$; ExC: $P = 0.94$
VCAM ng/mL, median (range)	494 (245-2,784)	419 (262-1,190)	417 (245-705)	518 (281-950)	473 (229-1,007)	462 (247-1,085)	455 (280-690)	495 (362-1,055)	E: $P = 0.37$; C: $P = 0.09$
VEGF ng/mL, median (range)	293 (0-588)	257 (0-399)	319 (0-957)	311 (0-812)	326 (0-516)	231 (17-422)	258 (56-863)	184 (12-613)	E: $P = 0.60$; C: $P = 0.31$

Abbreviations: E, exemestane; C, celecoxib; ExC, interaction.

*Ki67%, VCAM, VEGF: Mann-Whitney U tests; Apoptosis %, PR%: ANOVA exemestane versus placebo (group 3 and 4 versus 1 and 2); Ki67 median decrease 9% (95% CI, 6-14; $P = 0.001$). PR median reduction, 19% (95% CI, 9-28; $P = 0.011$). Celecoxib versus placebo Ki67 and PR, not significant.

greater than the 30% decrease we previously showed by preoperative withdrawal of HRT (14) and presumably reflects the very low serum concentrations of estradiol produced by exemestane compared with the usual postmenopausal estrogen concentrations seen after cessation of HRT.

Because approximately one third of DCIS are HER2 positive and ER and/or PR positive, this lesion provides an opportunity to compare the influence of HER2 expression on response to endocrine therapy because approximately two thirds of DCIS are HER2 positive. Although a decrease in Ki67 was seen with exemestane but not on placebo, the magnitude of the decrease was less in HER2-positive DCIS. Additionally, no reduction in PR expression was seen. Similar numbers of tumors had a 50% decrease in proliferation regardless of HER2 expression but HER2-positive DCIS had higher proliferation rates at baseline and at surgery. The lack of change in PR in HER2-positive DCIS may reflect the low numbers of HER 2 DCIS expressing PR before treatment.

Transgenic mice overexpressing COX-2 have reduced apoptosis in the breast epithelium and an increased rate of breast carcinogenesis (15). COX-2 catalyses key steps in the metabolism of arachnidonic acid to prostaglandin E2 and eicosanoids and is commonly overexpressed in DCIS, producing resistance to apoptosis through induction of the inhibitor of apoptosis protein survivin in cells (11).

Celecoxib is a potent inhibitor of COX-2 in animal models inducing increased apoptosis in tumors (11). However, no effect on apoptosis or proliferation in DCIS was seen with celecoxib, even in those tumors that expressed COX-2. Although we cannot exclude a small effect of celecoxib in this study, the likelihood of a clinically significant effect being missed is small. Preoperative studies of COX-2 inhibition with celecoxib in ER-negative DCIS have also failed to show an effect on proliferation or apoptosis (16). It is notable that aromatase inhibition was effective in preventing proliferation in HER2-positive and HER2-negative tumors. Because 60% of DCIS are HER2 positive, there may be benefits to the use of an aromatase inhibitor in this population rather than using tamoxifen.

A preclinical and small phase II feasibility study of the combination of exemestane with celecoxib suggested that combination therapy might increase response to exemestane in advanced breast cancer (17). Two larger recent phase III randomized trials have found no advantage to the combination in response rates (18, 19). One trial compared letrozole with exemestane alone or in combination with celecoxib but letrozole had the highest response rate, although it did not reach significance compared with the combination arm (19).

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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