original report

Celecoxib With Neoadjuvant Chemotherapy for Breast Cancer Might Worsen Outcomes Differentially by COX-2 Expression and ER Status: Exploratory Analysis of the REMAGUSO2 Trial

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abstract

PURPOSE The overexpression of cyclooxygenase 2 (COX-2) gene, also known as prostaglandin-endoperoxide synthase 2 (*PTGS2*), occurs in breast cancer, but whether it affects response to anticox drugs remains unclear. We investigated the relationships between *PTGS2* expression, celecoxib use during neoadjuvant chemotherapy (NAC), and both event-free survival (EFS) and overall survival (OS).

MATERIALS AND METHODS We analyzed a cohort of 156 patients with human epidermal growth factor receptor 2–negative breast cancer from the REMAGUS02 (ISRCTN Registry No. 10059974) trial with pretreatment *PTGS2* expression data. Patients were treated by sequential NAC (epirubicin plus cyclophosphamide followed by docetaxel with or without celecoxib). Experimental validation was performed on breast cancer cell lines. The Cancer and Leukemia Group B (CALGB) 30801 (ClinicalTrials.gov identifier: NCT01041781) trial that tested chemotherapy with or without celecoxib in patients with lung cancer served as an independent validation cohort.

RESULTS After 94.5 months of follow-up, EFS was significantly lower in the celecoxib group (hazard ratio [HR], 1.7; 95% CI, 1 to 2.88; $P = .046$). A significant interaction between *PTGS2* expression and celecoxib use was detected ($P_{interaction} = .01$). In the *PTGS2*-low group (n = 100), EFS was lower in the celecoxib arm (HR, 3.01; 95% CI, 1.45 to 6.24; $P = .002$) than in the standard treatment arm. Celecoxib use was an independent predictor of poor EFS, distant relapse–free survival, and OS.

Celecoxib in addition to docetaxel enhanced cell viability in *PTGS2*-low cell lines but not in *PTGS2*-high cell lines. In CALGB 30801, a trend toward poorer progression-free survival was observed in the patients with low urinary metabolite of prostaglandin E2 who received celecoxib (HR = 1.57; 95% CI, 0.87 to 2.84; $P = .13$).

CONCLUSION Celecoxib use during chemotherapy adversely affected survival in patients with breast cancer, and the effect was more marked in *PTGS2*-low and/or estrogen receptor–negative tumors. COX-2 inhibitors should preferably be avoided during docetaxel use in patients with breast cancer who are undergoing NAC.

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ASSOCIATED CONTENT

Appendix

Data Supplement

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

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INTRODUCTION

Cyclooxygenase-2 (COX-2; also known as PTGS2 [prostaglandin-endoperoxide synthase 2]) is an isoform of the key enzyme in eicosanoid biosynthesis *PTGS*, which catalyzes the rate-limiting step in prostaglandin synthesis. COX-2 overexpression has been observed in various malignant tumors, including lung,[1] colon,[2] and breast[3,4] cancers. Preclinical studies have shown that COX-2 overexpression and the resulting production of prostaglandins stimulated angiogenesis and proliferation, which promoted cell invasion and metastasis development.[5,6] High COX-2 levels are associated with poor outcome in many tumor models and clinical studies.[7-9] However, there is no consensus about the prognostic or predictive value of COX-2 expression in invasive breast carcinoma.[10-12]

The selective COX-2 inhibitor celecoxib was released onto the market in 2000 for the symptomatic treatment of arthritis. Celecoxib binds reversibly to a hydrophilic pocket near the active site of COX-2 and thus inhibits the conversion of arachidonic acid to prostaglandin H2. This results in anti-inflammatory and pain-relieving effects. Selective COX-2 inhibitors have also been explored as therapeutic or preventive agents in various oncologic settings.[13,14] Several studies have evaluated celecoxib in the neoadjuvant setting for breast cancer as a monotherapy[15,16] or combined with endocrine therapy.[17,18] In addition to toxicity and safety

concerns, the benefits of such strategies to patients with breast cancer were not sufficiently high for these agents to be incorporated into standard care, and the development of COX-2 inhibitors in oncology thus fell short of initial expectations.[19,20]

The REMAGUS02 (ISRCTN Registry No. 10059974) study was a multicenter, randomized, phase II trial that included 340 patients with locally advanced breast cancer. Patients were randomly assigned to receive neoadjuvant sequential chemotherapy (NAC; either epirubicin plus cyclophosphamide, followed by docetaxel alone or docetaxel plus celecoxib [400 mg twice per day orally] for human epidermal growth factor receptor 2 (*HER2*)–negative tumors [n = 220]; or docetaxel alone or docetaxel plus trastuzumab for *HER2*-positive tumors [n = 120]). The trial found no benefit of celecoxib in terms of pathologic complete response[21] (primary objective) or disease-free survival[22] (DFS; secondary objective).

Predictive biomarkers are biologic indicators of the likely response of a patient to a particular drug. Estrogen receptor (ER), progesterone receptor, and *HER2* status, which are used to determine the potential benefits of endocrine and trastuzumab treatments, are currently the only predictive markers used in clinical settings in breast cancer. However, many patients still do not respond to these therapies, and the identification of additional biomarkers to provide personalized treatment to population subgroups remains an important task in breast oncology.

In this study, we investigated the dependence of the effects of celecoxib on COX-2 expression by performing a post hoc exploratory analysis of the REMAGUS02 trial to evaluate survival as a function of *PTGS2* expression, as assessed by reverse transcription quantitative polymerase chain reaction (RT-qPCR). We validated our findings experimentally on breast cancer cell lines, and we performed analyses in an independent cohort of patients with non–small-cell lung cancer (NSCLC) from the Cancer and Leukemia Group B (CALGB) 30801 (ClinicalTrials.gov identifier: NCT01041781).

MATERIALS AND METHODS

Patients

In total, 220 patients with locally advanced breast cancer were included in the *HER2*-negative stratum of the REMAGUS02 phase II randomized trial. The patients received sequential chemotherapy with, first, epirubicin plus cyclophosphamide alone followed by docetaxel with or without celecoxib 400 mg administered twice per day orally with random assignment to arm 1 (without celecoxib) or arm 2 (with celecoxib), as previously described.[21,22] The full protocol (REMAGUS02 protocol; Appendix, online only), CONSORT diagram (Appendix Fig A1, online only), and results of the clinical trial (REMAGUS02 trial; Appendix) are provided. The use of celecoxib was suspended by the French Health Products Safety Agency from December 2004 to September 2005 because of safety concerns. Thereafter, the use of this agent was authorized but with a revision of the informed consent form. As a result, 13 patients randomly assigned to the celecoxib group did not receive this drug. Analyses of the results of this study were performed on an intention-to-treat basis and per-protocol analyses are provided in the Appendix. For the 220 patients who were randomly assigned, 156 (71%) had frozen pretreatment biopsy specimens that contained more than 30% invasive epithelial tumor cells and that were available for RT-qPCR analysis (raw data in Data Supplement). Among them, 139 patients had Affymetrix U133A chips (Thermo Fisher Scientific, Waltham, MA) with baseline gene expression data available (standard treatment, n = 72; celecoxib, n = 67).

PTGS2 (COX-2) Expression

Total RNA extraction from frozen pretreatment biopsy specimens, reverse transcription, and qPCR analysis and quality control were performed as previously described.[23,24] The *RPLPO*, TATA box-binding protein (*TBP*), transferrin receptor (*TFR*), beta-actin, beta-glucuronidase (*GUS*), and *GAPDH* genes were used as endogenous reference genes. Target quantities were normalized relative to the median value for the six reference genes. No consensus threshold has been defined for RT-qPCR analyses, so *PTGS2* gene expression was classified on the basis of tertiles (low, intermediate, and high). The odds ratios (ORs) for pathologic complete response of tertiles 1 (OR, 1; four [7.7%] of 52); and 2 (OR, 0.77; three [6%] of 50) were essentially similar (*v* OR, 4.22; 13 [26%] of 50 for tertile 3), so we chose to merge those two tertiles (*PTGS2*-low) and compare them with the third tertile (*PTGS2*-high), as previously described.[24]

Statistical Analysis

To investigate if tumors were different between the celecoxib and noncelecoxib arms, we performed a differential expression analysis between the two groups of treatment (Appendix). Event-free survival (EFS) was defined as the time from random assignment to progression, locoregional recurrence, distant recurrence, or death, whichever occurred first. Distant relapse–free survival (DRFS) was defined as the time from random assignment to first distant metastasis or death; overall survival (OS) was defined as the time from random assignment to death. Patients for whom none of these events was recorded were censored at the date of last known contact. The cutoff date for the analysis was May 1, 2015. Predictive effects were evaluated with a test of interaction between treatment group and *PTGS2* expression and ER status. EFS and OS were estimated using the Kaplan-Meier method, and survival curves were compared using a log-rank test. Univariable Cox proportional hazard models were performed to determine the variables associated with survival. Covariables selected for the multivariable analysis were those with *P* values no greater than .15 after univariable analysis. A multivariable

TABLE 1. Patient and Tumor Characteristics at Baseline by Treatment Arm in the Intention-to-Treat Population

Characteristic	Standard Treatment Arm (n = 78)	Celecoxib Arm (n = 78)	*P*
Age, years			
< 40	17 (21.8)	17 (21.8)	.55
40 to 49	30 (38.5)	36 (46.2)	
≥ 50	31 (39.7)	25 (32.1)	
Menopausal status			
Pre	51 (66.2)	55 (70.5)	.69
Post	26 (33.8)	23 (29.5)	
Mean BMI, kg/m^2	25.8 (5.0)	24.5 (4.7)	.09
Tumor size			
T2	40 (51.3)	49 (62.8)	.2
T3 and T4	38 (48.7)	29 (37.2)	
Clinical nodal status			
N0	30 (39.0)	29 (37.2)	.95
N1, N2, N3	47 (61.0)	49 (62.8)	
Histology			
Ductal	67 (85.9)	64 (82.1)	.77
Lobular	7 (9.0)	8 (1.3)	
Other	4 (5.1)	6 (7.7)	
Grade			
1	9 (12.2)	5 (6.6)	.22
2	25 (33.8)	35 (46.1)	
3	40 (54.1)	36 (47.4)	
LVI			
No	67 (85.9)	64 (83.1)	.8
Yes	11 (14.1)	13 (16.9)	
ER status			
Negative	27 (34.6)	29 (37.2)	.87
Positive	51 (65.4)	49 (62.8)	
PR status			
Negative	42 (54.5)	44 (57.9)	.8
Positive	35 (45.5)	32 (42.1)	
TNBC			
Yes	26 (33.3)	29 (37.2)	.74
No	52 (66.7)	49 (62.8)	
p53			
WT	20 (54.1)	24 (61.5)	.67
Mutated	17 (45.9)	15 (38.5)	
Surgery			
No	2 (2.6)	2 (2.6)	.99
Yes	76 (97.4)	76 (97.4)	
Adjuvant chemotherapy			
No	54 (69.2)	55 (70.5)	.99
Yes	24 (3.8)	23 (29.5)	
Endocrine therapy			
No	23 (31.1)	25 (34.2)	.82
Yes	51 (68.9)	48 (65.8)	
Radiotherapy			
No	1 (1.3)	3 (4.1)	.59
Yes	74 (98.7)	70 (95.9)	

NOTE. Data are presented as No. (%). The following data are missing: menopausal status (n = 1), BMI (n = 1), clinical nodal status (n = 1), grade (n = 6), LVI (n = 1), PR (n = 3), *p53* (n = 80), endocrine therapy (n = 9), radiotherapy (n = 8), and pCR (n = 4).

Abbreviations: BMI, body mass index; ER, estrogen receptor; LVI, lymphovascular invasion; PR, progesterone receptor; TNBC, triple-negative breast cancer; WT, wild type.

model was then implemented using a forward stepwise selection procedure. Analyses were performed with R software, version 3.1.2.

Experimental Validation and Independent Human Validation Cohort

We performed an experimental validation on two *PTGS2*-low breast cancer cell lines (MDA-MB-231 and MDA-MB-157), and two *PTGS2*-high cell lines (BT549 and MDA-MB-436; Appendix). Cell lines were treated with increasing concentrations of docetaxel with or without celecoxib 25 μM. Cellular viability was assessed at 72 hours. Statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software, San Diego, CA). The data were expressed as the mean and standard error of the mean (SEM). One-way analyses of variance followed by Bonferroni post hoc comparison tests were performed in all statistical analyses. The results were considered statistically significant at a $P < .05$, $P < .01$, or $P < .001$. To confirm our results, we also performed a post hoc reanalysis of the CALGB 30801 trial,[25] in which 312 patients with advanced NSCLC were randomly assigned to receive celecoxib or placebo in addition to standard chemotherapy. We stratified the analyses by the expression levels of the urinary after they were stratified by the expression levels of the urinary metabolite of prostaglandin E2 (PGE-M; Appendix).

RESULTS

Analyses of the REMAGUS02 Trial

Patient population. In total, 156 patients from the REMAGUS02 trial were included in this study; 78 were randomly assigned to the celecoxib arm, and 78 were randomly assigned to the arm with standard treatment only. Patient and tumor baseline characteristics were similar in the celecoxib and standard treatment arms (Table 1). In addition, no gene of 19,965 was differentially expressed

TABLE 2. EFS and OS HRs by Celecoxib Use for the Whole Study Population, the ER-Negative Subpopulation, and the ER-Positive Subpopulation of the Intention-to-Treat Analyses

	Whole Population (n = 156)					ER Negative (n = 56)					ER Positive (n = 100)				
Survival by Population	**No. of Patients**	**No. of Events**	**HR**	**95% CI**	**$P_{Log\text{-}Rank}$**	**No. of Patients**	**No. of Events**	**HR**	**95% CI**	**$P_{Log\text{-}Rank}$**	**No. of Patients**	**No. of Events**	**HR**	**95% CI**	**$P_{Log\text{-}Rank}$**
EFS															
Whole population															
Standard treatment arm	77	23	1		.046	27	7	1		.027	50	16	1		.523
Celecoxib arm	78	35	1.7	1 to 2.88		29	15	2.69	1.08 to 6.71		49	20	1.24	0.64 to 2.39	
PTGS2 low (n = 104)															
Standard treatment arm	49	10	1		.002	12	1	1		.002	37	9	1		.121
Celecoxib arm	54	27	3.01	1.45 to 6.24		12	9	13.45	1.68 to 107.44		42	18	1.87	0.84 to 4.16	
PTGS2 high (n = 52)															
Standard treatment arm	28	13	1		.52	15	6	1		.971	13	7	1		.331
Celecoxib arm	24	8	0.75	0.3 to 1.83		17	6	0.98	0.32 to 3.04		7	2	0.46	0.09 to 2.29	
OS															
Whole population															
Standard treatment arm	77	14	1		0.108	27	6	1		.027	50	8	1		.97
Celecoxib arm	78	23	1.71	0.88 to 3.33		29	14	2.84	1.08 to 7.47		49	9	1.02	0.39 to 2.64	
PTGS2 low (n = 104)															
Standard treatment arm	49	5	1		.012	12	1	1		.001	37	4	1		.434
Celecoxib arm	54	17	3.32	1.23 to 9.01		12	9	13.64	1.71 to 108.87		42	8	1.61	0.48 to 5.35	
PTGS2 high (n = 52)															
Standard treatment arm	28	9	1		.668	15	5	1		.931	13	4	1		.38
Celecoxib arm	24	6	0.8	0.28 to 2.24		17	5	0.95	0.27 to 3.27		7	1	0.39	0.04 to 3.48	

Abbreviations: EFS, event-free survival; ER, estrogen receptor; HR, hazard ratio; OS, overall survival.

between the celecoxib arm and the standard treatment arm, consistent with the random allocation of patients to the celecoxib arm.

Notable differences in tumor characteristics according to *PTGS2* status were observed. The frequencies of grade III, *p53*-mutated, ER-negative and progesterone receptor–negative tumors were higher in the *PTGS2*-high population than in the *PTGS2*-low population (Appendix Table A1, online only).

The Effect of Celecoxib on Survival is Modified by *PTGS2* Expression and ER Status

EFS analysis. In the full study cohort of patients with *HER2*-negative disease (n = 156), celecoxib use was significantly associated with shorter EFS (hazard ratio [HR], 1.7; 95% CI, 1 to 2.88; $P = .046$; Table 2). There was a significant interaction between *PTGS2* expression and celecoxib for EFS ($P_{interaction} = .01$), which meant that the effect of celecoxib on EFS differed significantly between the *PTGS2*-low and *PTGS2*-high groups.

In the *PTGS2*-low group, celecoxib use was associated with shorter EFS (HR, 3.01; 95% CI, 1.45 to 6.24; $P = .002$; Fig 1A), and the obtained results differed by ER status. In ER-negative tumors, celecoxib use was strongly associated with shorter EFS (HR, 13.45; 95% CI, 1.68 to 107.44; $P = .002$; Fig 1B), whereas celecoxib had no effect on EFS in ER-positive tumors (HR, 1.87; 95% CI, 0.84 to 4.16; $P = .121$; $P_{interaction} = .02$; Fig 1C). In the *PTGS2*-high group, celecoxib use did not affect EFS (Fig 1D) in either the ER-negative (Fig 1E) or ER-positive (Fig 1F) population.

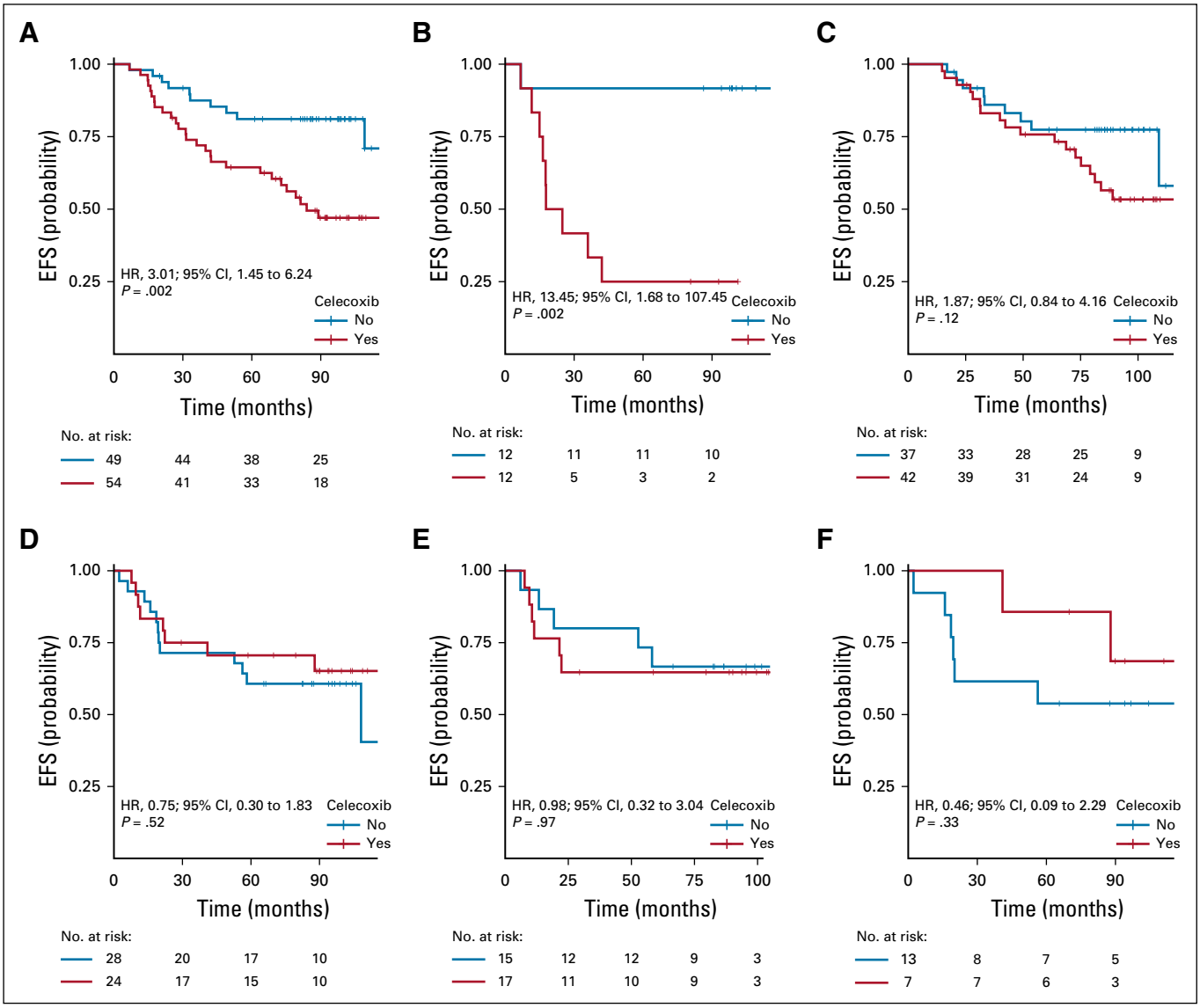

FIG 1. Kaplan-Meier curves for association between treatment arm and event-free survival (EFS), according to *PTGS2* and estrogen receptor (ER) status: (A) *PTGS2*-low population; (B) *PTGS2*-low/ER-negative subpopulation; (C) *PTGS2*-low/ER-positive subpopulation; (D) *PTGS2*-high population; (E) *PTGS2*-high/ER-negative subpopulation; and (F) *PTGS2*-high/ER-positive subpopulation. HR, hazard ratio.

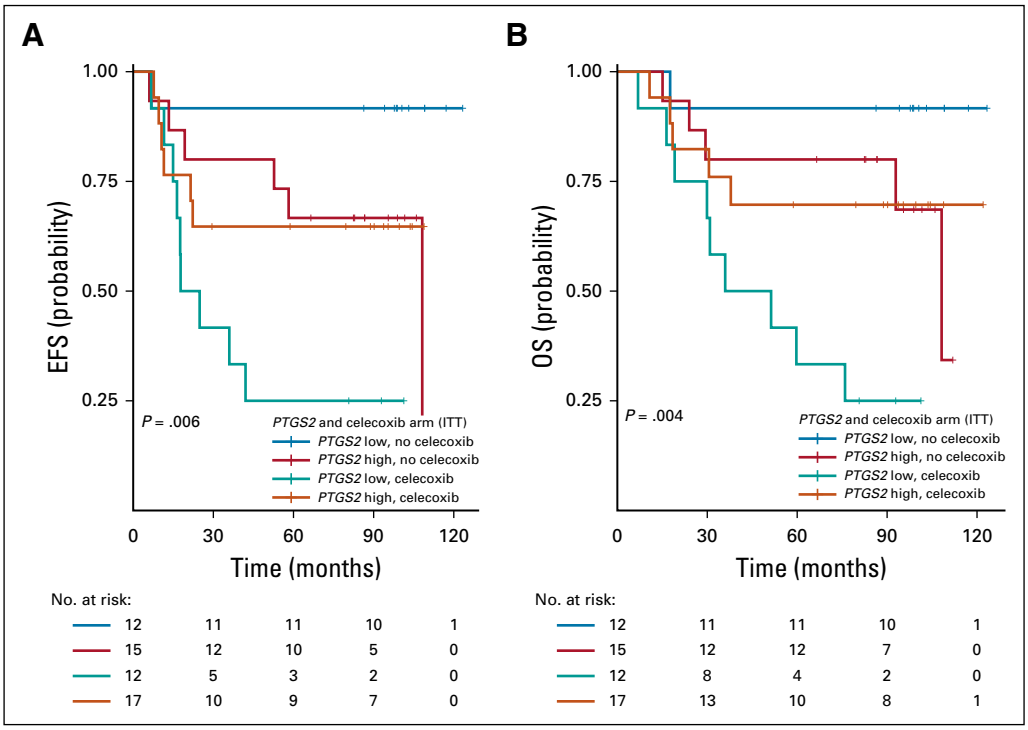

FIG 2. Kaplan-Meier combined survival curves for the association between *PTGS2* expression and treatment arm in the estrogen receptor (ER)–negative population. (A) Event-free survival (EFS) by *PTGS2* expression and celecoxib use; (B) overall survival (OS) by *PTGS2* expression and celecoxib use. ITT, intention to treat.

The association between celecoxib use and impaired EFS ($P < .001$), the interactions between celecoxib use and *PTGS2* expression ($P = .008$), and the interactions between celecoxib use and ER status ($P = .005$) were highly significant after multivariable analysis (Appendix Table A2, online only). Similar results were also found for DRFS (data not shown).

OS analyses. Similar results were obtained for OS (Table 2). In the *PTGS2*-low group, celecoxib use was associated with a shorter OS (HR, 3.32; 95% CI, 1.23 to 9.01; $P = .012$; Appendix Fig A2A, online only), and its effects differed according to ER status ($P_{interaction} = .05$). Celecoxib use was associated with a shorter OS in ER-negative tumors (HR, 13.64; 95% CI, 1.71 to 108.87; $P = .001$; Appendix Fig A2B) but had no significant effect on OS in ER-positive tumors (HR, 1.61; 95% CI, 0.48 to 5.35; $P = .434$; Appendix Fig A2C).

In the *PTGS2*-high group, celecoxib use had no effect on OS (Appendix Fig A2D) in the ER-negative population (Appendix Fig A2E) or in the ER-positive population (Appendix Fig A2F).

The association between celecoxib use and impaired OS ($P = .001$), the interactions between celecoxib use and *PTGS2* expression ($P = .03$), and the interactions between celecoxib use and ER status ($P = .02$) were again significant after multivariable analysis (Appendix Table A3, online only). The combined Kaplan-Meier curves for EFS and OS as a function of *PTGS2* expression and celecoxib use are shown for ER-negative tumors in Figure 2.

Per-protocol analyses. Analyses of this study on a per-protocol basis showed comparable results that are provided in the Appendix (Appendix Table A4, online only; Appendix Figs A3, A4, and A5, online only).

Experimental validation. **The addition of celecoxib to docetaxel enhances cell viability in PTGS2-low but not in PTGS2-high breast cancer cell lines.** To assess whether preclinical models would mimic the clinical observations, we performed translational research by studying a panel of four ER-negative and *HER2*-negative breast cancer cell lines. *PTGS2* expression was very low in MDA-MB-231 and MDA-MB-157, whereas it was high in BT549 and MDA-MB-436 (Appendix Fig A6, online only). In all four triple-negative breast cancer cell lines, celecoxib alone (5 to 200 μM) had no effect on cellular viability (data not shown).

In the *PTGS2*-low cell lines (MDA-MB-231 and MDA-MB-157), addition of celecoxib enhanced cellular viability compared with docetaxel treatment alone (Figs 3A and 3B). In *PTGS2*-high cell lines (BT549 and MDA-MB-436), celecoxib in association with docetaxel had no effect on cellular viability (Figs 3C and 3D). These cell culture results therefore match the clinical observations and suggest the following: (1) The effect of celecoxib in addition to chemotherapy varies with the expression levels of *PTGS2*, and this effect is restricted to *PTGS2*-low cell lines. (2) In

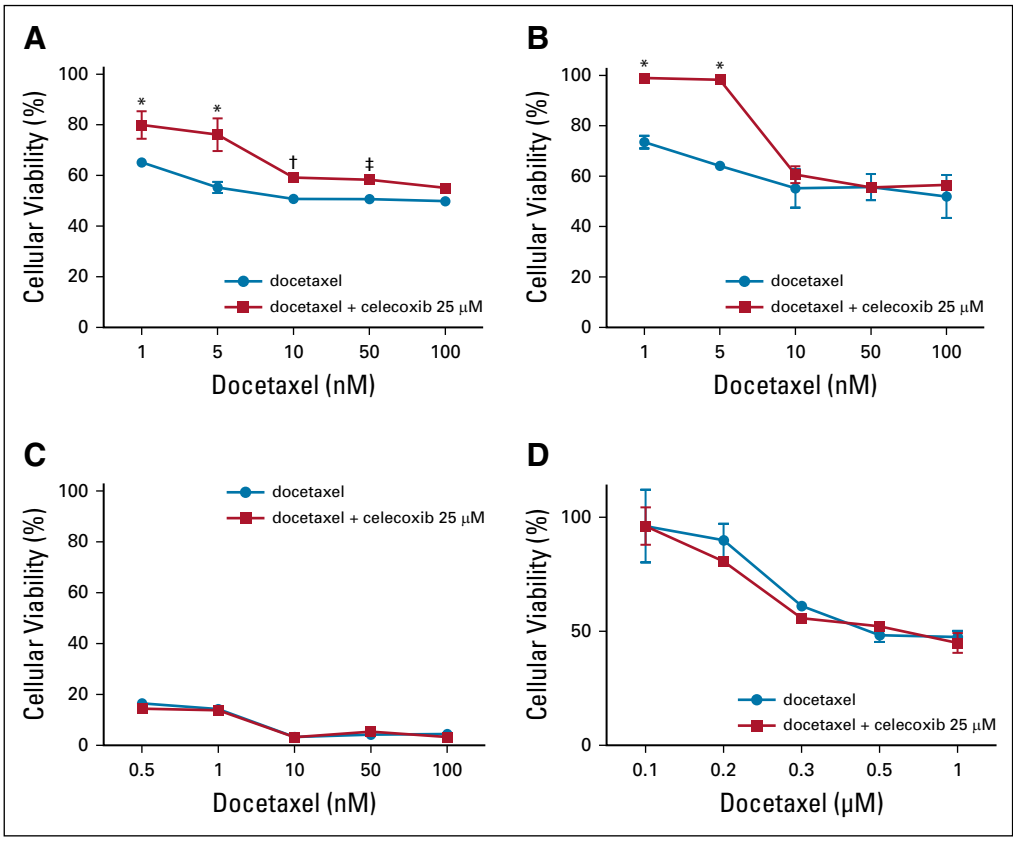

FIG 3. Effect of docetaxel alone or in combination with celecoxib on cellular viability in *PTGS2*-low cell lines (A) MDA-MB-231 and (B) MDA-MB-157 as well as *PTGS2*-high cell lines (C) BT549 and (D) MDA-MB-436. (*) $P < .001$; (†) $P < .01$; (‡) $P < .05$.

PTGS2-low cell lines, the addition of celecoxib to taxanes enhances cellular viability compared with taxanes alone.

Analyses of the CALGB 30801 trial. **The effect of celecoxib in addition to chemotherapy is associated with a trend toward an impaired progression-free survival in patients with NSCLC who have low values of PGE-M.** In the population of the CALGB 30801 trial with metabolite of prostaglandin E2 (PGE-M) data available, the addition of celecoxib to chemotherapy had no impact on PFS (celecoxib *v* no celecoxib: HR, 1.08; 95% CI, 0.85 to 1.36; $P = .53$). In the population with PGE-M values less than quartile 1 (Q1), celecoxib in addition to chemotherapy was associated with a trend toward impaired progression-free survival (PFS) compared with chemotherapy alone (HR, 1.57; 95% CI, 0.87 to 2.84; $P = .13$). In contrast, for the population with PGE-M values of Q1 or greater, the addition of celecoxib to chemotherapy was not associated with differences in PFS (HR, 0.91; 95% CI, 0.66 to 1.26; $P = .57$; Appendix Figs A7A and A7B, respectively, online only).

DISCUSSION

In this exploratory analysis of the REMAGUS02 trial, we report an adverse effect of celecoxib use during NAC on survival in patients with breast cancer. The magnitude of this effect was greater in patients with either *PTGS2*-low tumors or ER-negative tumors, and it was particularly dramatic in the subgroup of patients with ER-negative and *PTGS2*-low tumors. One might have expected COX-2 inhibitors to act preferentially on tumors cells that express COX-2. Instead, we identified a paradoxical effect on cells with a low expression of *PTGS2*. The clinical observation was reproduced experimentally by performing translational research in four different breast cancer cell lines. Importantly, this effect was observed only in combination with taxanes and not with celecoxib alone. These results are particularly important because despite the evidence of a potential protective effect of nonsteroidal anti-inflammatory drugs (NSAIDs) against breast cancer in preclinical and epidemiologic data, no randomized trial, to our knowledge, has investigated the addition of any NSAID to NAC in breast cancer. Previous or unpublished randomized trials have been designed using celecoxib alone,[26] but evidence is still lacking for the effects of celecoxib in addition to NAC in humans.[27] We also found a trend toward a similar effect in an independent cohort derived from a randomized clinical trial, in a different setting, and in another cancer localization (advanced NSCLC).

These results raise concerns about the safety of COX-2 inhibitors during chemotherapy in patients with breast cancer. They are consistent with a recent study[11] performed on a cohort of 911 patients with breast cancer, which identified an interaction among COX-2 expression, prognosis, and preoperative NSAID use ($P_{interaction} = .009$). In that study, patients with preoperative NSAID treatment

TABLE 3. Summary of Randomized Controlled Trials to Evaluate Combinations of COX-2 Inhibitors With Chemotherapy in Patients With Cancer

First Author	Setting and Cancer Type	COX-2 Assessment	No. of Patients in the Analyses	Conclusion of the Authors	Premature or Temporary Discontinuation	Primary End Point	Comments	Interaction/Stratification by COX-2 expression (when assessed)
Maiello[30]	First-line locally advanced and/or metastatic colorectal cancer	No	FOLFIRI (n = 38) or FOLFIRI + CBX (n = 39)	FOLFIRI regimen was effective and well tolerated as first-line treatment in patients with advanced colorectal cancer. The addition of CBX to the FOLFIRI regimen did not improve results.		ORR	The ORR was lower in the arm with combined CBX. ORR: FOLFIRI *v* FOLFIRI + CBX: 45% (95% CI, 29% to 61%) *v* 36% (95% CI, 21% to 51%)	No
Kohne[31]	First-line metastatic colorectal cancer	No	FOLFIRI (n = 41) or CAPIRI (n = 44) with CBX (n = 42) or placebo (n = 43)	Because of the small sample size after early termination, no definitive conclusions could be drawn in relation to the noninferiority of CAPIRI compared with FOLFIRI.	Yes	PFS	Median PFS and OS times were shorter for CAPIRI *v* FOLFIRI (PFS: 5.9 *v* 9.6 months; OS: 14.8 *v* 19.9 months) and CBX *v* placebo (PFS: 6.9 *v* 7.8 months; OS: 18.3 *v* 19.9 months).	Assumptions of an absence of interaction between FU *v* capecitabine and CBX *v* placebo effects.
Jin[35]	First-line metastatic colorectal cancer	Yes (IHC)	FOLFOX4 (n = 30) *v* FOLFOX4 + CBX (n = 58)	The addition of CBX to the FOLFOX4 regimen increased the short-term efficacy and the 3-year survival rate.		Not reported	RR (CR + PR) was significantly greater in the group with FOLFOX4 + CBX than in the group with FOLFOX4 (P = .022)	No stratification
Lilenbaum[32]	Second-line treatment of stage IIIB or IV NSCLC	No	Irinotecan docetaxel (n = 69) + irinotecan gemcitabine (n = 64) with CBX (n = 67) or without CBX (n = 66)	CBX did not seem to enhance efficacy or improve patient-reported symptoms.	Yes	Median/1-year survival probabilities	Median survival of patients was higher with chemotherapy alone *v* with CBX: 8.99 months (95% CI, 6.60 to 11.14 months) *v* 6.31 months (95% CI, 4.53 to 8.57 months).	Study design assumed no interaction between chemotherapy treatment and use of CBX.
Gridelli[36]	First-line treatment stage IIIB or IV NSCLC	No	Gemcitabine IV (n = 200) or PCI + cisplatin (n = 200) with rofecoxib (n = 149) or without rofecoxib (n = 251)	Rofecoxib improved RR but did not prolong survival. The trial was closed prematurely because of safety issues.	Yes	OS		The study was not planned to test efficacy interactions in the experimental factors.
Edelman[28]	First-line treatment stage IIIB or IV NSCLC	Yes (IHC, n = 83)	Carboplatin + gemcitabine + CBX (n = 44) + zileuton (n = 45) + CBX + zileuton (n = 45)	This study failed to demonstrate the value of dual eicosanoid inhibition or benefit from either agent alone in addition to chemotherapy.		9-month failure-free survival	CBX treatment associated with a trend toward worse OS outcome (HR, 1.59; 95% CI, 0.85 to 2.96; P = .15) after multivariable analysis.	Interaction of receiving CBX and COX-2 expression on OS (P = .0026); analyses stratified by COX-2 expression
Groen[34]	First-line treatment stage IIIB or IV NSCLC	Yes (31%)	Docetaxel carboplatin with CBX (n = 281) or placebo (n = 280)	In advanced NSCLC, CBX did not improve survival.		OS		Interaction between COX-2 expression and the impact on CBX/placebo treatment was tested but was not significant. Analyses were stratified by COX-2 expression.

(continued on following page)

TABLE 3. Summary of Randomized Controlled Trials to Evaluate Combinations of COX-2 Inhibitors With Chemotherapy in Patients With Cancer (continued)

First Author	Setting and Cancer Type	COX-2 Assessment	No. of Patients in the Analyses	Conclusion of the Authors	Premature or Temporary Discontinuation	Primary End Point	Comments	Interaction/Stratification by COX-2 expression (when assessed)
Koch[37]	First-line treatment stage IIIB or IV NSCLC	No	Palliative chemotherapy with CBX (n = 158) or placebo (n = 158)	This study failed to demonstrate a survival benefit of the addition of CBX to palliative chemotherapy.	Yes	OS	In women, survival was shorter with placebo than with CBX (HR, 1.16; 95% CI, 0.83 to 1.62), whereas the opposite was observed in men (HR, 0.79; 95% CI, 0.57 to 1.09).	No
Edelman[29]	Second-line treatment stage IIIB or IV NSCLC	Yes, baseline urinary PGE-M	Docetaxel or pemetrexed with apricoxib (n = 36) or placebo (n = 36)	Apricoxib did not improve PFS, despite biomarker-driven patient selection.		PFS	Patients who received docetaxel + apricoxib (n = 17) had a numerically inferior median PFS of 75 days (95% CI, 47 to 104 days) *v* 97 days (95% CI, 48 to 216 days) for those who received docetaxel + placebo (n = 20; HR, 1.62; *P* = .18)	Interaction between baseline PGE-M and chemotherapeutic agents (docetaxel *v* pemetrexed) for PFS (*P* = .026).
Edelman[25]	Second-line treatment stage IIIB or IV NSCLC	Yes (n = 312; COX-2 IHC and urinary PGE-M)	Carboplatin pemetrexed + gemcitabine with CBX (n = 154) or with placebo (n = 158)	COX-2 expression by IHC failed to select patients who could benefit from selective COX-2 inhibition.	Yes	PFS	Complementary analyses (unpublished, performed for this study): In patients with PGE-M values < Q1 (n = 53), there was a trend toward impaired PFS with CBX compared with CT alone (HR, 1.57; 95% CI, 0.87 to 2.84; *P* = .13).	Interaction between treatment effect (CBX *v* placebo) and baseline urinary PGE-M level significant for OS (*P* = .02) but not for PFS (*P* = .22)
Reyners[33]	First-line stage IC to IV ovarian cancer	Yes (61%; n = 120)	Carboplatin docetaxel ± CBX	CBX did not influence PFS and OS, but interpretation of results was hampered by premature CBX discontinuation.	Yes	RR and PFS	CBX use was associated with a trend toward worse PFS in the multivariable analysis (HR, 1.28; 95% CI, 0.90 to 1.81; *P* = .16).	No
This study	Neoadjuvant treatment of locally advanced breast cancers	Yes (*PTGS2* RT-qPCR; n = 156)	Epirubicin cyclophos-phamide followed by docetaxel (n = 78) + CBX (n = 78)	CBX was associated with impaired EFS (*P* = .05) and OS (*P* = .11), particularly in the *PTGS2*-low and the ER-negative groups.	Yes	pCR	CBX use associated with impaired EFS, metastasis-free survival, OS after multivariable analysis	Significant interactions between *PTGS2* expression and CBX use (*P* = .008) and ER status and CBX use (*P* = .005) on EFS

Abbreviations: CAPIRI, irinotecan plus capecitabine; CBX, celecoxib; COX-2, cyclooxygenase 2; CR, complete response; EFS, event-free survival; ER, estrogen receptor; FOLFIRI, fluorouracil, leucovorin, and irinotecan; FOLFOX4, folinic acid–fluorouracil– oxaliplatin; FU, fluorouracil; HR, hazard ratio; IHC, immunohistochemistry; IV, intravenously; NSCLC, non–small-cell lung cancer; ORR, objective response rate; OS, overall survival; PCI, prolonged constant infusion; pCR, pathologic complete response; PFS, progression-free survival; PGE-M, prostaglandin E_2 metabolite; PR, partial response; Q1, quartile 1; RR, response rate; RT-qPCR, reverse transcription quantitative polymerase chain reaction.

and COX-2-negative tumors had a significantly higher risk of events (HR, 4.51; $P < .001$) compared with the other patients.

Furthermore, several randomized trials that investigated multiple COX-2 inhibitors in addition to chemotherapy for the treatment of different cancers have reported interactions[25,28,29] among COX-2 inhibitor use, COX expression, chemotherapy regimen, and clinical outcome. The findings of these previous studies are listed in Table 3.[28-37] In eight of these studies[25,28-33] (including this study), COX-2 inhibitor use during chemotherapy was, or tended to be, associated with a poorer outcome than no COX-2 inhibitor use. Of note, seven trials were temporarily or prematurely discontinued because of safety concerns

or enrollment failure, which may partially explain underpowered definitive analyses. Although several regimens were used, evidence that the combination of COX-2 inhibitor with chemotherapy might be detrimental was reported in four trials (including this study) that evaluated taxane-based chemotherapy regimens.[29,32,33] Finally, only two of 12 randomized trials were stratified for COX-2 expression.[28,34] The re-analysis of the 10 remaining trials after stratification by COX-2 expression could unmask a hidden deleterious or beneficial effects in specific subgroups.

This study has limitations. The REMAGUS02 trial was a phase II randomized trial that was only designed to assess the efficacy of celecoxib in the whole population, but analyses stratified by ER or *PTGS2* status were not prespecified. Hence, we cannot strictly infer causality for the negative association we report in the subpopulations. However, two arguments suggest that the relevance of these subgroup analyses is not spurious. First, the interactions between COX-2 inhibitors and COX-2 expression has already been demonstrated by multiple teams.[25,28,29] Second, both ER status, which is a pivotal biomarker for any breast cancer trial, and *PTGS2* (COX-2 expression), which is the very target of the drug tested (ie, celecoxib), have a strong biologic rationale to justify these subgroup analyses. Finally, we cannot derive any information on the safety profile of celecoxib in *HER2*-positive tumors because of the design of the REMAGUS02 trial (none of the patients with *HER2*-positive disease received celecoxib). The safety data in *HER2*-positive tumors could have been informative, because Subbaramaiah et al[38] has reported that celecoxib can interrupt *HER2* downstream signaling.

This study also has several strengths. As the only randomized trial, to our knowledge, to assess celecoxib in association with NAC in patients with breast cancer, the results show independent, significant, negative associations with EFS, DRFS, and OS, after a long follow-up, both in the intention-to-treat and the per-protocol analyses. A validation phase III trial specifically powered to confirm the deleterious impact of this drug in specified subgroups would be unethical. Thus, these data will remain unique for the foreseeable future. Finally, because there cannot and will not be a confirmatory trial to establish strict causality between celecoxib use during NAC for breast cancer and the risk of adverse outcome, physicians should apply caution and recommend alternatives to prescriptions of celecoxib in patients with ER-negative, *HER2*-negative breast cancer who are being treated with taxane-containing NAC.

This study has several implications: (1) Given the hypothesis-generating value of these findings, additional research should be performed and may include the post hoc reanalysis of randomized trials that evaluated COX-2 inhibitors in addition to chemotherapy after stratification by COX-2 expression. We also strongly recommend that investigators of clinical trials that evaluate COX-2 inhibitors should provide individual patient data that could be pooled into large meta-analyses. Such an effort is critical to reach robust evidence to derive routine recommendations about the avoidance or the safety of the routine prescription of COX-2 inhibitors during chemotherapy. (2) Evidence for synergy between COX inhibitors and checkpoint blockade immunotherapy is emerging.[39,40] On the basis of our results, we recommend the stratification of all future trials that involve these inhibitors according to COX-2 expression status. (3) In the absence of other evidence, we recommend avoidance of celecoxib use and preference for alternative drugs in patients with ER-negative, *HER2*-negative breast tumors who are receiving docetaxel-containing NAC, unless the expected benefit greatly outweighs the potential risks. Only by carefully addressing these concerns will it be possible to determine the subgroups of patients most likely to benefit from COX inhibitors.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Celecoxib With Neoadjuvant Chemotherapy for Breast Cancer Might Worsen Outcomes Differentially by COX-2 Expression and ER Status: Exploratory Analysis of the REMAGUS02 Trial

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APPENDIX

Analysis of the REMAGUS02 Trial

Patients. All of the patients included in this study were informed about the study in advance and gave written consent for participation in the trial and ancillary studies (ISRCTN Registry No. 10059974, French ethics committee Paris-Bicêtre, No. 03-55). The primary outcome measure of the trial was pathologic complete response (pCR), evaluated according to Chevallier criteria.[21] The secondary outcome measures were the definition of genomic profiles of success (ie, pCR) or failure for each type of treatment, and these results have been published elsewhere, together with quality-control data.[23]

Samples. In total, 156 samples from the 220 patients with breast cancer were available for transcriptomic analyses. The subgroup of 156 patients with available reverse transcription quantitative polymerase chain reaction (RT-qPCR) data did not differ from the remaining 64 patients of the *HER2*-negative population in terms of age, menopausal status, clinical tumor size, or nodal involvement. However, lobular and grade 1 and 2 tumors were overrepresented in the population without available transcriptome data relative to the population with available transcriptome available (26.6% *v* 9.6% [$P = .004$] and 58.3% *v* 49.3% [$P = .03$], respectively). Raw data for the patients are provided in the Data Supplement.

Statistical Analysis

Differential expression analysis. Of 156 patients with RT-qPCR available for *PTGS2* expression, 139 had Affymetrix U133A chips (Thermo Fisher Scientific, Waltham, MA) available for analysis. We performed a differential analysis by comparing the mean gene expression of each group according to treatment arm (celecoxib *v* no celecoxib) using a linear model (limma R package) and retained as differentially expressed genes those for which the mean expression was different with a *P* value of .05 or lower. The analysis was performed in the whole population and after stratification by *PTGS2* status (*PTGS2*-low, n = 93; *PTGS2*-high, n = 46).

Experimental Validation

Cell lines. Human breast cancer cell lines BT-549, MDA-MB-436, MDA-MB-231, and MDA-MB-157 were obtained from the American Type Culture Collection (ATCC, Manassas, VA). The cell lines were authenticated every 20 passages using the GenePrint 10 system kit (B9510; Promega, Madison, WI). All cell lines were cultured in RPMI-1640 medium or DMEM (Thermo Fisher Scientific, Waltham, MA) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific) and 1% antibiotics (penicillin 50 μg/mL, streptomycin 50 μg/mL, neomycin 100 μg/mL; Thermo Fisher Scientific), at 37°C in a humidified atmosphere that contained 5% CO_2.

Drugs. Docetaxel was purchased from Téva laboratory (Courbevoie, France). Celecoxib was purchased from Biogaran laboratory (Colombes, France) and was dissolved in phosphate-buffered saline.

Viability assay. A total of 8,000 cells per well were seeded in P96 plates and allowed to adhere for 24 hours at 37°C. Cells were then treated with various concentrations of chemotherapeutic agents and/or celecoxib for 72 hours. Cellular proliferation was measured using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay according to the manufacturer's instructions (Promega, Madison, WI). Absorbance was measured at 490 nm on a 96-well microplate reader (Dynatech Laboratories MRX, Chantilly, VA).

Experimental plan. We assessed the in vitro antitumor activity of celecoxib in combination with chemotherapeutic agents on the triple-negative breast cancer cell lines. MDA-MB-231 and MDA-MB-157 were defined by qRT-PCR as *PTGS2*-low cell lines; BT549 and MDA-MB-436, as *PTGS2*-high cell lines. To this end, we evaluated cellular viability under increasing concentrations of docetaxel in combination or not with celecoxib 25μM.

Analyses of the Cancer and Leukemia Group P 30801 trial. To confirm our results, we performed a post hoc reanalysis of the Cancer and Leukemia Group B 30801 Alliance trial.[21] In this trial, 312 patients with in advanced non–small-cell lung cancer—stage IIIB with pleural effusion or stage IV—were randomly assigned to receive celecoxib or placebo in addition to standard chemotherapy. Only patients with a COX-2 index of two or greater were registered and randomly assigned to treatment. Urinary metabolite of prostaglandin E2 (PGE_2), hereafter designated PGE-M, was evaluated at the baseline and on day 8 of the first cycle in 211 patients in the study. Patients were evenly divided into four groups (quartiles) that were based on the quantity of urinary PGE-M at baseline (Q1, 10.09; Q2, 15.38; and Q3, 27.86 ng/mg creatinine). Progression-free survival was analyzed according to celecoxib addition, and the Q1 cutoff was used for PGE-M (PGE-M $<$ Q1 *v* PGE-M $\geq$ Q1). Kaplan-Meier curves were used for survival analysis, and the log-rank test was used to assess differences in progression-free survival between PGE-M–defined patient groups.

Per-Protocol Analyses Results: Survival Analyses—The Effect of Celecoxib on Survival is Modified by *PTGS2* Expression and Estrogen Receptor Status

Event-free survival analysis. In the *PTGS2*-low group, celecoxib use was associated with poorer event-free survival (EFS; hazard ratio [HR], 1.96; 95% CI, 1.02 to 3.76; $P = .039$; 8-year EFS: 50.5% [95% CI, 37.3% to 68.4%] *v* 73.1% [95% CI, 62.3% to 85.8%]; Appendix Table A4, online only; Appendix Fig A3A, online only), but the obtained results differed according to ER status ($P_{interaction} = .011$). In ER-negative tumors, celecoxib use was associated with poor EFS (HR, 7.18; 95% CI, 1.5 to 34.3; $P = .004$, Appendix Fig A3B, online only), whereas it had no such effect on EFS in ER-positive tumors ($P = .65$; Appendix Fig A3C, online only). In the *PTGS2*-high group, celecoxib use was not associated with EFS (Appendix Fig A3D, online only), in either the ER-negative (Appendix Fig A3E, online only) or the ER-positive (Appendix Fig A3F, online only) population.

Overall survival analysis. Similar results were obtained for overall survival (OS; Appendix Table A3, online only; Appendix Figs A4A through A4F, online only). Celecoxib use was associated with poor OS in the *PTGS2*-low/ER-negative subgroup (HR, 6.81; 95% CI, 1.43 to 32.33; $P = .005$; 8-year OS, 27.3% [95% CI, 10.4% to 71.6%] *v* 84.6% [95% CI, 67.1% to 100%]; Appendix Fig A3B online only) but not in the *PTGS2*-low/ER-positive subgroup (HR, 0.81; 95% CI, 0.26 to 2.56; $P = .72$; $P_{interaction(celecoxib/ER\ status)} = .02$; Appendix Fig A4C online only). Finally, Appendix Figure A5 shows the Kaplan-Meier curves for EFS and OS according to *PTGS2* expression and the effect of celecoxib in ER-negative tumors in per-protocol analyses.

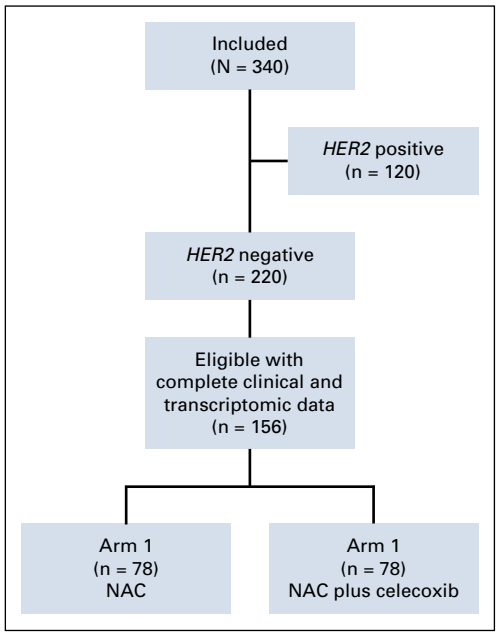

FIG A1. Study flow diagram of included patients and tumors samples available for reverse transcription quantitative polymerase chain reaction analysis in the REMAGUS02 (ISRCTN Registry No. 10059974) biologic trial. NAC (neoadjuvant chemotherapy [epirubicin + cyclophosphamide followed by docetaxel]); *HER2*, human epidermal growth factor receptor 2.

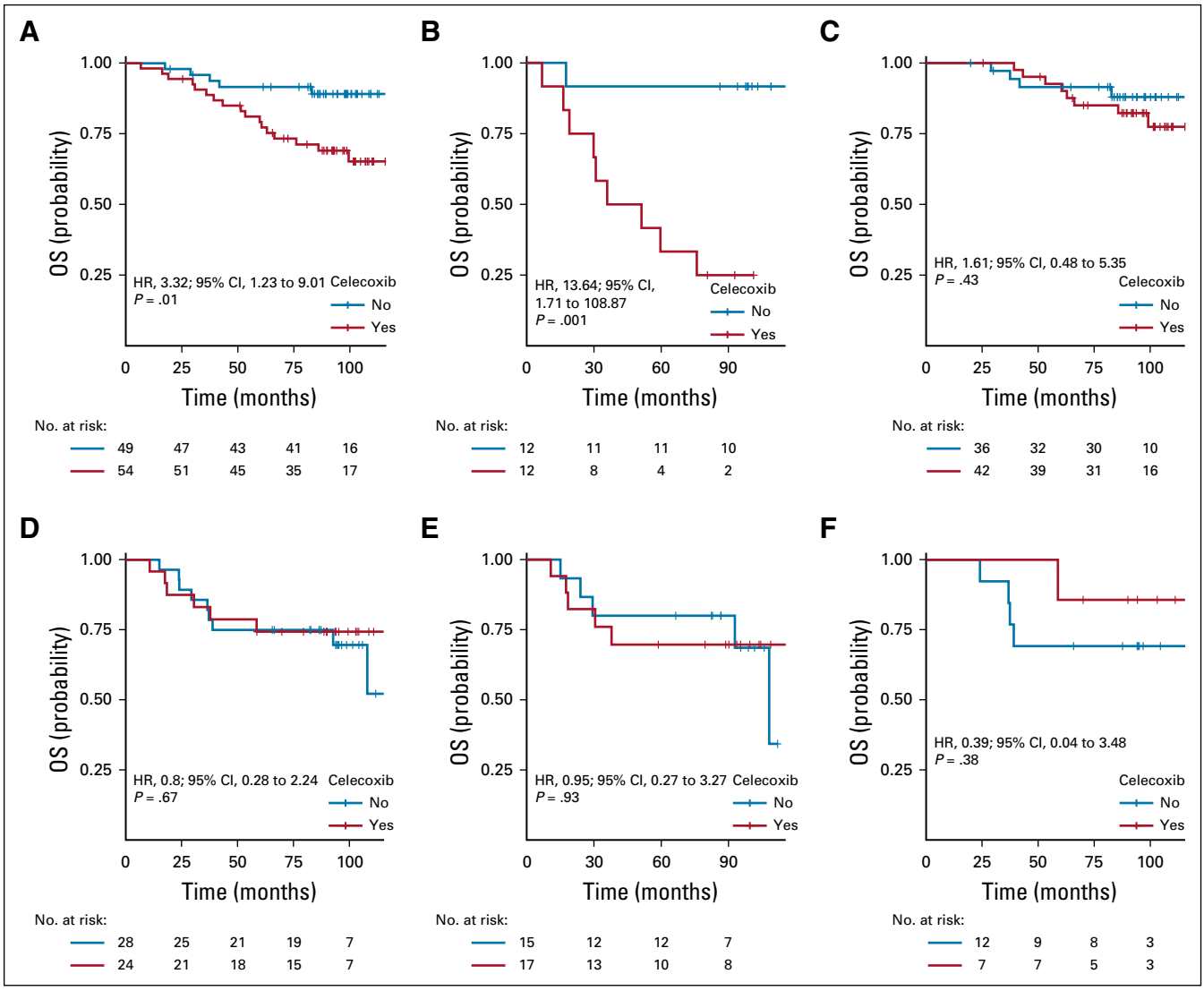

FIG A2. Kaplan-Meier curves for association between treatment arm (intention-to-treat analyses) and overall survival (OS) according to *PTGS2* expression and estrogen receptor (ER) status: (A) *PTGS2*-low population; (B) *PTGS2*-low/ER-negative subpopulation; (C) *PTGS2*-low/ER-positive subpopulation; (D) *PTGS2*-high population; (E) *PTGS2*-high/ER-negative subpopulation; and (F) *PTGS2*-high/ER-positive subpopulation. HR, hazard ratio.

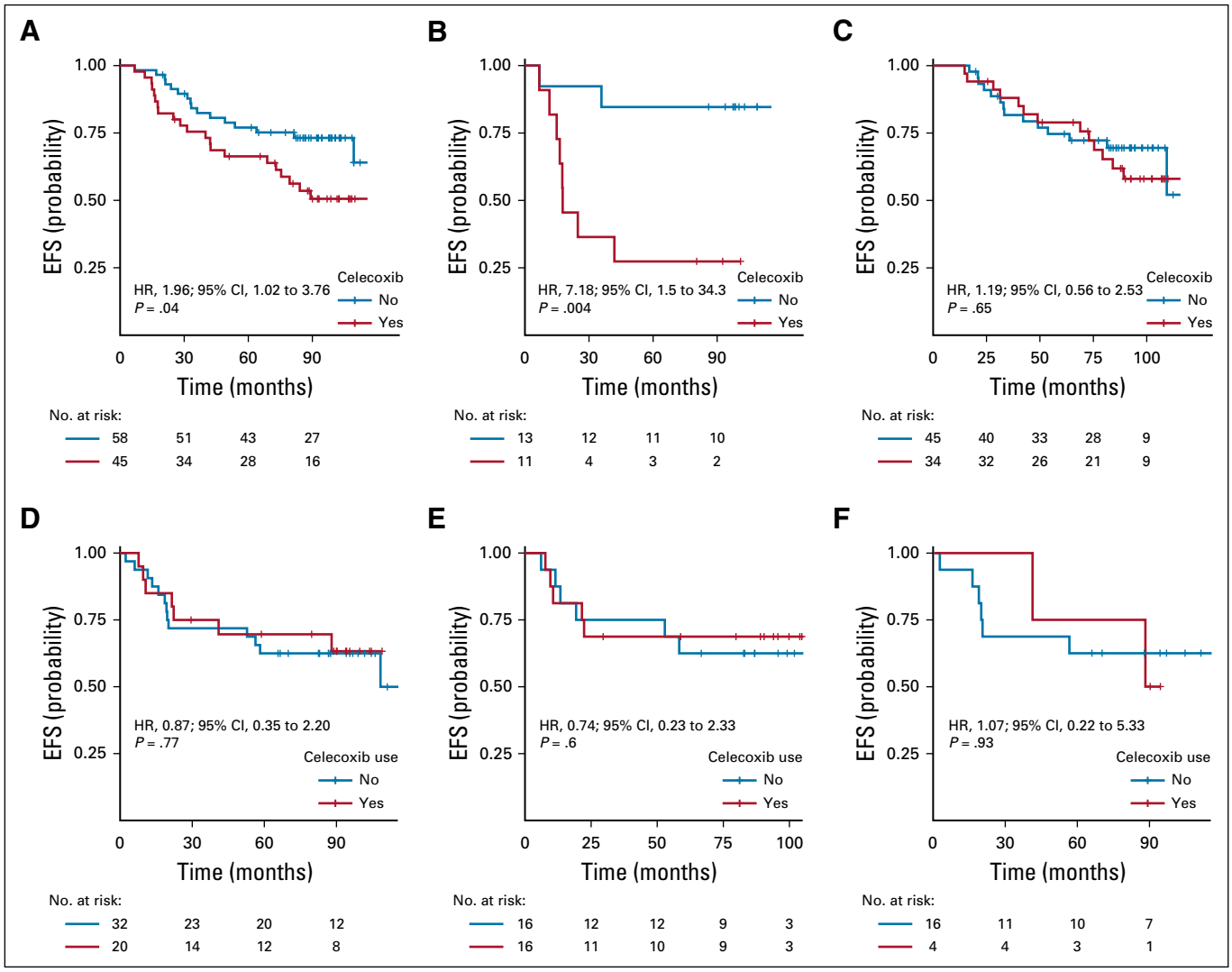

FIG A3. Kaplan-Meier curves for association between treatment arm (per-protocol analyses) and event-free survival (EFS) according to *PTGS2* and estrogen receptor (ER) status: (A) *PTGS2*-low population; (B) *PTGS2*-low/ER-negative subpopulation; (C) *PTGS2*-low/ER-positive subpopulation; (D) *PTGS2*-high population; (E) *PTGS2*-high/ER-negative subpopulation; and (F) *PTGS2*-high/ER-positive subpopulation. HR, hazard ratio.

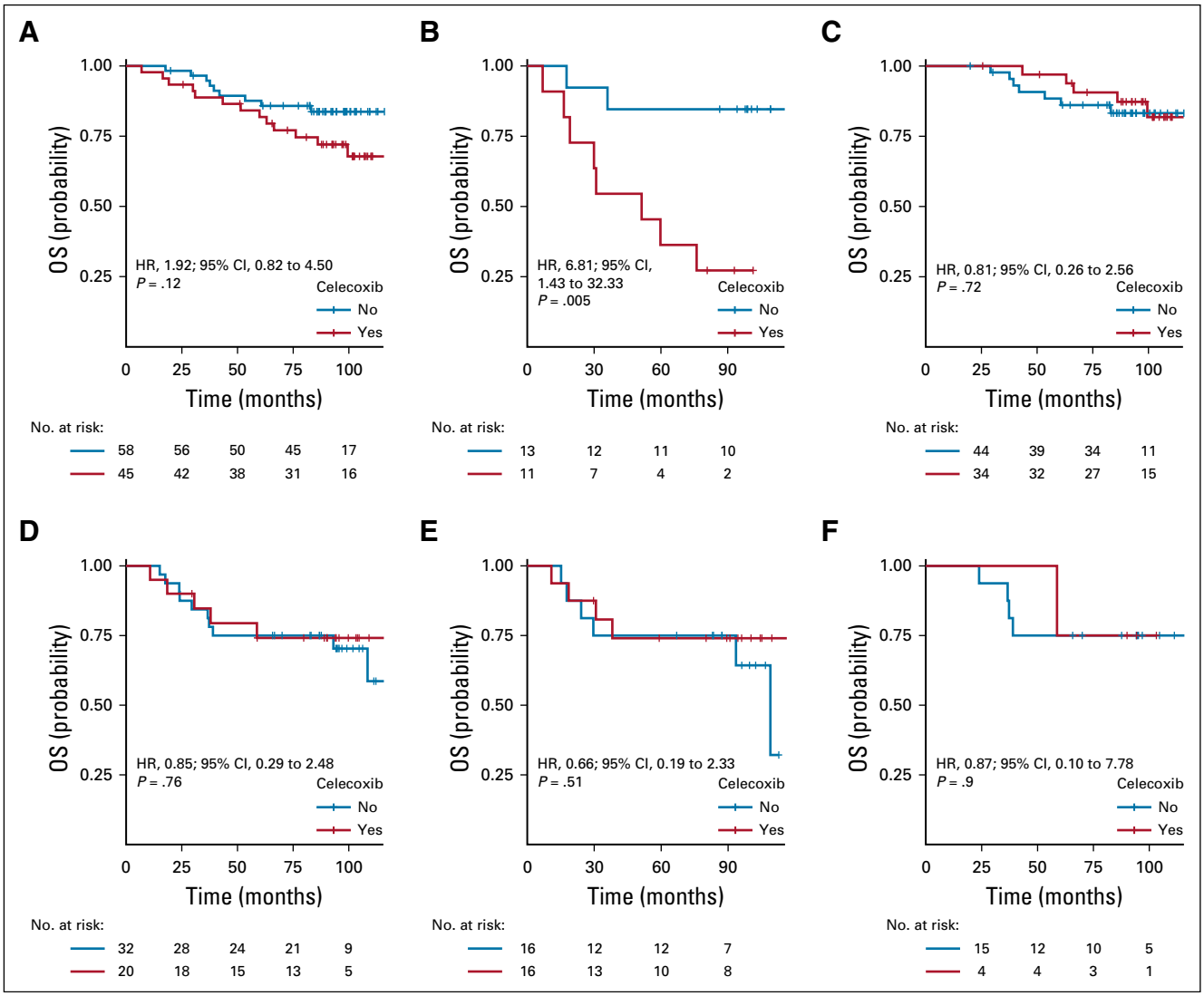

FIG A4. Kaplan-Meier curves for association between treatment arm (per-protocol analyses) and overall survival (OS) according to *PTGS2* expression and estrogen receptor (ER) status: (A) *PTGS2*-low population; (B) *PTGS2*-low/ER-negative subpopulation; (C) *PTGS2*-low/ER-positive subpopulation; (D) *PTGS2*-high population; (E) *PTGS2*-high/ER-negative subpopulation; and (F) *PTGS2*-high/ER-positive subpopulation. HR, hazard ratio.

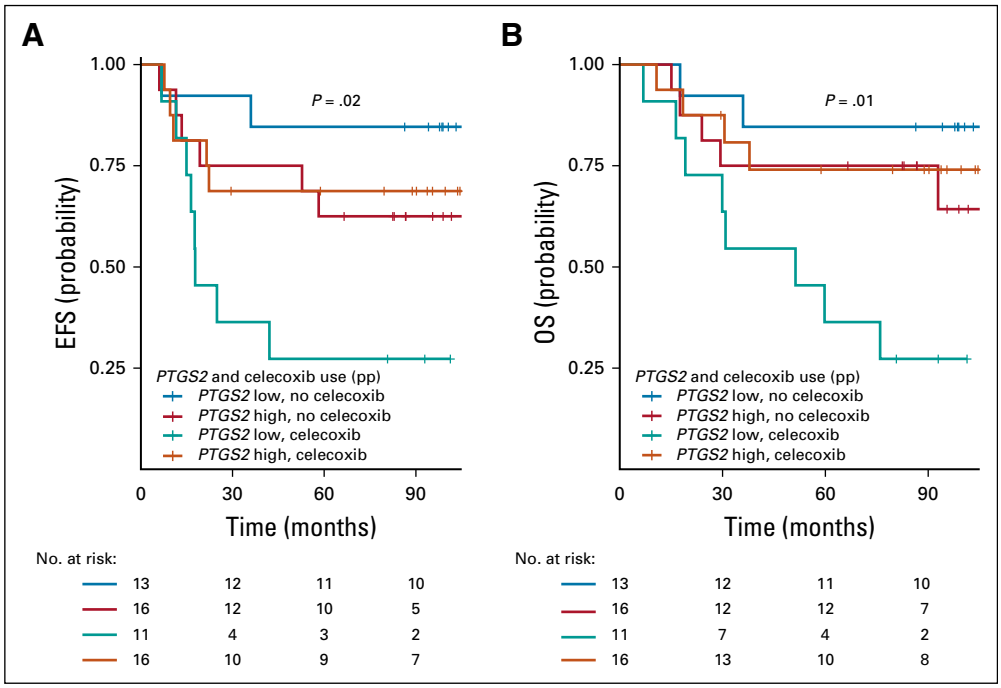

FIG A5. Kaplan-Meier combined survival curves for the association between *PTGS2* expression and treatment arm (per-protocol analyses) in the estrogen receptor (ER)–negative population: (A) event-free survival (EFS) by *PTGS2* expression and celecoxib use; (B) overall survival (OS) by *PTGS2* expression and celecoxib use. pp, per protocol.

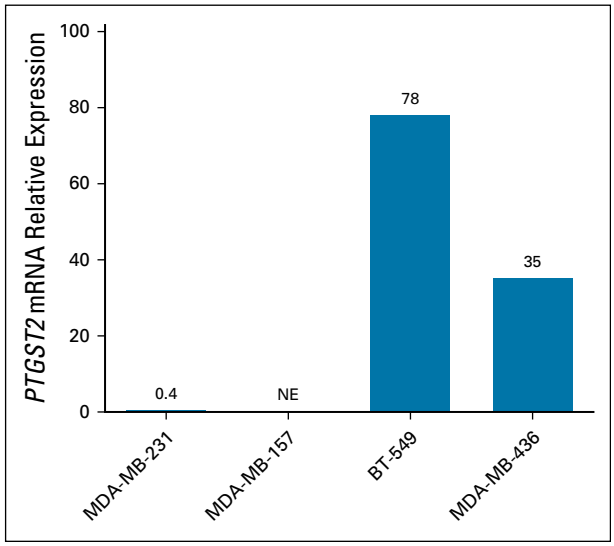

FIG A6. *PTGS2* expression by reverse transcription quantitative polymerase chain reaction analysis in four triple-negative breast cancer cell lines (MDA-MB-231; MDA-MB-157; BT549; and MDA-MB-436). NE, not expressed.

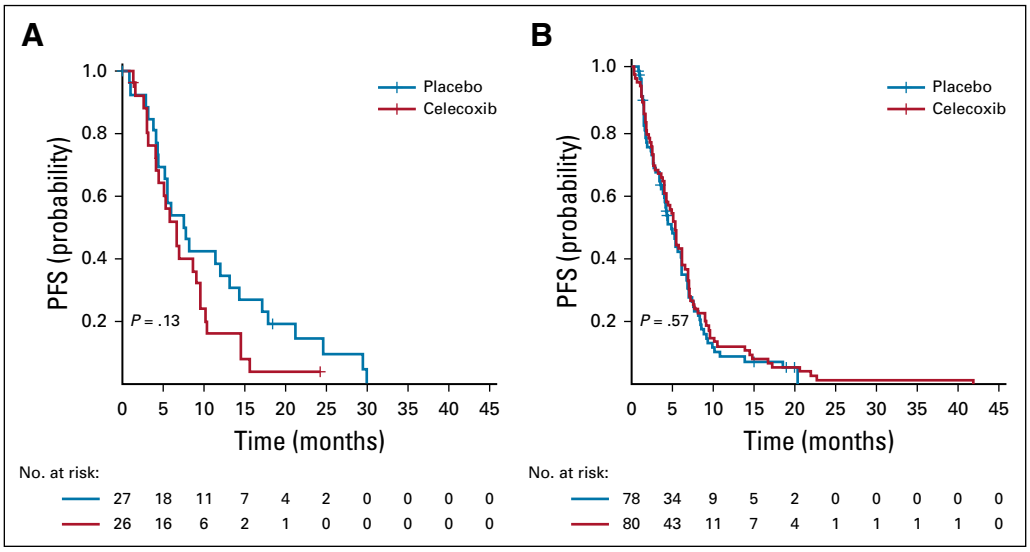

FIG A7. Kaplan-Meier curves for association between treatment arm and progression-free survival (PFS) in the Cancer and Leukemia Group B 30801 Alliance trial: (A) population with metabolite prostaglandin E_2 (PGE-M) values less than Q1 (less than first quartile); and (B) population with PGE-M values of Q1 or greater (≥ first quartile).

TABLE A1. Patient and Tumor Characteristics by *PTGS2* Expression in the Intention-to-Treat Population

Characteristic	No. (%) of Patients		P
	***PTGS2* Low (n = 104)**	***PTGS2* High (n = 52)**	
Age, years			
< 40	23 (22.1)	11 (21.2)	.99
40 to 49	44 (42.3)	22 (42.3)	
≥ 50	37 (35.6)	19 (36.5)	
Menopausal status			
Pre	68 (66.0)	38 (73.1)	.48
Post	35 (34.0)	14 (26.9)	
BMI, kg/m^2			
≤ 25	60 (57.7)	28 (54.9)	.88
> 25	44 (42.3)	23 (45.1)	
Tumor size			
T2	62 (59.6)	27 (51.9)	.46
T3 and T4	42 (4.4)	25 (48.1)	
Clinical nodal status			
N0	37 (35.6)	22 (43.1)	.46
N1, N2, N3	67 (64.4)	29 (56.9)	
Histology			
Ductal	88 (84.6)	43 (82.7)	.11
Lobular	12 (11.5)	3 (5.8)	
Other	4 (3.8)	6 (11.5)	
Grade			
1	12 (11.7)	2 (4.3)	< .01
2	48 (46.6)	12 (25.5)	
3	43 (41.7)	33 (70.2)	
LVI			
No	88 (85.4)	43 (82.7)	.83
Yes	15 (14.6)	9 (17.3)	
ER status			
Negative	24 (23.1)	32 (61.5)	< .01
Positive	80 (76.9)	20 (38.5)	
PR status			
Negative	47 (46.1)	39 (76.5)	< .01
Positive	55 (53.9)	12 (23.5)	
TNBC			
Yes	23 (22.1)	32 (61.5)	< .01
No	81 (77.9)	20 (38.5)	
p53			
WT	35 (71.4)	9 (33.3)	< .01
Mutated	14 (28.6)	18 (66.7)	
Celecoxib (pp)			
No	59 (56.7)	32 (61.5)	.69
Yes	45 (43.3)	20 (38.5)	

Abbreviations: BMI, body mass index; ER, estrogen receptor; LVI, lymphovascular invasion; pp, per protocol; PR, progesterone receptor; TNBC, triple-negative breast cancer; WT, wild type.

TABLE A2. Univariable and Multivariable Analyses of Clinical and Pathologic Factors on EFS

	Univariable			Multivariable		
Variable Comparison	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age, years						
40 to 49 *v* < 40	0.73	0.37 to 1.42	.428			
≥ 50 *v* < 40	1.05	0.54 to 2.06				
Menopausal status						
Post *v* pre	0.91	0.52 to 1.61	.755			
Tumor size						
T3 and T4 *v* T2	1.97	1.17 to 3.31	.009			
Clinical nodal status						
N1, N2, and N3 *v* N0	1.49	0.84 to 2.62	.169			
Histology						
Lobular *v* ductal or other	2.13	1.16 to 3.92	.013			
Grade						
3 *v* 2	1.19	0.69 to 2.06	.524			
ER status						
Positive *v* negative	0.79	0.46 to 1.35	.386	1.04	0.4 to 2.69	.931
PR status						
Positive *v* negative	0.54	0.31 to 0.94	.026			
LVI						
Yes *v* no	1.93	1.04 to 3.6	.035	2.26	1.14 to 4.48	.02
Treatment allocation						
Celecoxib arm *v* standard arm	1.7	1 to 2.88	.046	9.17	2.88 to 29.15	< .001
PTGS2 expression						
High *v* low	1.24	0.73 to 2.13	.425	2.50	1.05 to 5.95	.038
pCR status						
Yes *v* no	0.33	0.1 to 1.06	.051	0.21	0.06 to 0.75	.016
Interaction: celecoxib with *PTGS2*			.011			.008
Interaction: celecoxib with ER			.106			.005

Abbreviations: EFS, event-free survival; ER, estrogen receptor; HR, hazard ratio; LVI, lymphovascular invasion; pCR, pathologic complete response PR, progesterone receptor.

TABLE A3. Univariable and Multivariable Analyses of Clinical and Pathologic Factors on OS

	Univariable			Multivariable		
Variable Comparison	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age, years						
40 to 49 *v* < 40	0.79	0.33 to 1.92	.379			
≥ 50 *v* < 40	1.33	0.57 to 3.1				
Menopausal status						
Post *v* pre	0.94	0.46 to 1.91	.866			
Tumor size						
T3 and T4 *v* T2	2.8	1.43 to 5.5	.002	2.7653	1.35 to 5.67	.006
Clinical nodal status						
N1, N2, and N3 *v* N0	1.46	0.72 to 2.96	.286			
Histology						
Lobular *v* ductal or other	2.39	1.18 to 4.86	.013			
Grade						
3 *v* 2	1.51	0.76 to 3.02	.239			
ER status						
Positive *v* negative	0.41	0.21 to 0.78	.005	1.86	0.6 to 5.81	.283
PR status						
Positive *v* negative	0.25	0.11 to 0.57	< .001	0.3049	0.11 to 0.81	.017
LVI						
Yes *v* no	2.04	0.96 to 4.34	.058			
Treatment allocation						
Celecoxib arm *v* standard arm	1.71	0.88 to 3.33	.108	9.75	2.41 to 39.45	.001
PTGS2 expression						
High *v* low	1.47	0.76 to 2.83	.25	2.32	0.73 to 7.35	.154
pCR status						
Yes *v* no	0.37	0.09 to 1.55	.158			
Interaction: celecoxib with *PTGS2*			.045			.03
Interaction: celecoxib with ER			.124			.023

Abbreviations: ER, estrogen receptor; HR, hazard ratio; LVI, lymphovascular invasion; OS, overall survival; pCR, pathologic complete response; PR, progesterone receptor.

TABLE A4. EFS and OS HRs in Per-Protocol Analyses by Celecoxib Use and *PTGS2* Expression in the Whole Study Population, the ER-Negative Subpopulation, and the ER-Positive Subpopulation

	Whole Population (n = 156)					ER Negative (n = 56)					ER Positive (n = 100)				
Survival by Population	**No. of Patients**	**No. of Events**	**HR**	**95% CI**	$P_{Log\text{-}Rank}$	**No. of Patients**	**No. of Events**	**HR**	**95% CI**	$P_{Log\text{-}Rank}$	**No. of Patients**	**No. of Events**	**HR**	**95% CI**	$P_{Log\text{-}Rank}$
EFS															
Whole population															
Standard treatment arm	90	30	1		.168	29	9	1		.116	61	21	1		.815
Celecoxib arm	65	28	1.44	0.86 to 2.41		27	13	1.97	0.83 to 4.66		38	15	1.08	0.56 to 2.1	
PTGS2 low (n = 104)															
Standard treatment arm	58	16	1		.039	13	2	1		.004	45	14	1		.65
Celecoxib arm	45	21	1.96	1.02 to 3.76		11	8	7.18	1.5 to 34.3		34	13	1.19	0.56 to 2.53	
PTGS2 high (n = 52)															
Standard treatment arm	32	14	1		.773	16	7	1		.605	16	7	1		.933
Celecoxib arm	20	7	0.87	0.35 to 2.2		16	5	0.74	0.23 to 2.33		4	2	1.07	0.22 to 5.33	
OS															
Whole population															
Standard treatment arm	90	19	1		.349	29	8	1		.138	61	11	1		.596
Celecoxib arm	65	18	1.36	0.71 to 2.59		27	12	1.95	0.79 to 4.81		38	6	0.76	0.28 to 2.07	
PTGS2 low (n = 104)															
Standard treatment arm	58	9	1		.125	13	2	1		.005	45	7	1		.72
Celecoxib arm	45	13	1.92	0.82 to 4.5		11	8	6.81	1.43 to 32.33		34	5	0.81	0.26 to 2.56	
PTGS2 high (n = 52)															
Standard treatment arm	32	10	1		.759	16	6	1		.511	16	4	1		.899
Celecoxib arm	20	5	0.85	0.29 to 2.48		16	4	0.66	0.19 to 2.33		4	1	0.87	0.1 to 7.78	

Abbreviations: EFS, event-free survival; ER, estrogen receptor; HR, hazard ratio; OS, overall survival.