Chromosome 17 Centromere Duplication and Responsiveness to Anthracycline-Based Neoadjuvant Chemotherapy in Breast Cancer[1,2]

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Abstract

Human epidermal growth factor receptor 2 (*HER2*) and *topoisomerase II alpha* (*TOP2A*) genes have been proposed as predictive biomarkers of sensitivity to anthracycline chemotherapy. Recently, chromosome 17 centromere enumeration probe (CEP17) duplication has also been associated with increased responsiveness to anthracyclines. However, reports are conflicting and none of these tumor markers can yet be considered a clinically reliable predictor of response to anthracyclines. We studied the association of *TOP2A* gene alterations, *HER2* gene amplification, and CEP17 duplication with response to anthracycline-based neoadjuvant chemotherapy in 140 patients with operable or locally advanced breast cancer. *HER2* was tested by fluorescence *in situ* hybridization and *TOP2A* and CEP17 by chromogenic *in situ* hybridization. Thirteen patients (9.3%) achieved pathologic complete response (pCR). *HER2* amplification was present in 24 (17.5%) of the tumors. *TOP2A* amplification occurred in seven tumors (5.1%). CEP17 duplication was detected in 13 patients (9.5%). CEP17 duplication correlated with a higher rate of pCR [odds ratio (OR) 6.55, 95% confidence interval (95% CI) 1.25-34.29, $P = .026$], and analysis of *TOP2A* amplification showed a trend bordering on statistical significance (OR 6.97, 95% CI 0.96-50.12, $P = .054$). *TOP2A* amplification and CEP17 duplication combined were strongly associated with pCR (OR 6.71, 95% CI 1.66-27.01, $P = .007$). *HER2* amplification did not correlate with pCR. Our results suggest that CEP17 duplication predicts pCR to primary anthracycline-based chemotherapy. CEP17 duplication, *TOP2A* amplifications, and *HER2* amplifications were not associated with prognosis.

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Introduction

Predicting response to anthracycline-based therapy is a central challenge in patients with breast cancer. Several randomized studies have shown that anthracycline-based adjuvant therapy produces a modest improvement in survival in patients with early-stage breast cancer [1]. In addition, it has been shown that incorporation of taxanes further improves pathologic complete response (pCR) in the neoadjuvant setting [2–7]. However, the risk of serious side effects must be considered and predictive factors are needed to help clinicians select the most appropriate drug for each patient. To date, no specific biomarkers have been identified to predict tumor response to anthracycline-based chemotherapy.

The *human epidermal growth factor receptor 2* (*HER2*) and *topoisomerase II alpha* (*TOP2A*) genes have been proposed as markers of sensitivity to anthracycline chemotherapy. Although several studies have reported an association between *HER2* amplification with anthracycline sensitivity [8–13], only two of these were statistically significant [10,13]. *In vitro* and *in vivo* studies indicate that *HER2* positivity alone does not alter anthracycline sensitivity [14] and the underlying mechanism remains elusive. The *TOP2A* gene, located at 17q12-q21, close to the *HER2* gene, encodes TOP2A, a key enzyme in DNA replication and the molecular target of anthracyclines [15,16]. When *HER2* is amplified, genes situated around 17q21, such as *TOP2A* gene, may be either co-amplified or deleted [17]. Because of this physical proximity, some researchers have proposed that the link between *HER2*-positive disease and anthracycline sensitivity is the presence of *TOP2A* alterations (amplifications and deletions) rather than *HER2* amplifications [18,19]. However, the results of these studies have not always been consistent [20].

Given the location of *HER2* and *TOP2A* on chromosome 17, recent reports have focused on the predictive role of other molecular alterations localized within the 17q21 region, including alterations in key genes such as *HER1-3* [21], *p53* [22], and *BRCA1* [23], and variations in the copy number of subchromosomal regions, including the chromosome 17 centromere (CEP17) duplication [21]. On the basis of recent data from array comparative genomic hybridization, CEP17 duplication is defined as increased copy number of CEP17 [21] rather than polysomies of the whole chromosome 17 [24,25]. CEP17 duplication has been described as a marker of genomic instability [26] and has received a great deal of attention as a potential predictor of anthracycline benefit [21,27].

We hypothesized that CEP17 duplication is associated with a higher response to neoadjuvant chemotherapy. We explored the association of CEP17 duplication and *TOP2A* alterations with response to anthracycline-based neoadjuvant therapy. We studied a cohort of patients with early or locally advanced breast cancer treated with anthracycline-based primary chemotherapy, and we chose pCR as a surrogate marker of chemosensitivity.

Materials and Methods

Ethics Statement

This study was conducted according to the Declaration of Helsinki principles, with approval from the Clinical Research Ethics Committee at Institut d'Investigacions Biomèdiques Sant Pau. Written informed consent was obtained from all patients.

Study Design and Patients

We retrospectively studied 140 consecutive patients with stage II or III breast cancer who received anthracycline-based neoadjuvant chemotherapy in our hospital between 1993 and 2010. All patients had confirmed diagnosis based on histopathology of biopsy and none of them had prior treatment with surgery, chemotherapy, or radiation. Our study included patients with *HER2*-positive carcinomas treated before the approval of trastuzumab in 2006. The study excluded patients with bilateral or inflammatory tumors. Fifty-five patients received neoadjuvant treatment with anthracyclines alone [FEC75: fluorouracil (600 mg/m^2), epirubicin (75 mg/m^2), and cyclophosphamide (600 mg/m^2) given every 3 weeks for four to six cycles, n = 40, or FAC60: fluorouracil (500 mg/m^2), doxorubicin (60 mg/m^2), and cyclophosphamide (500 mg/m^2) given every 3 weeks for four to six cycles, n = 15] between 1993 and 2002. Eighty-five patients received neoadjuvant treatment with anthracyclines in combination with taxanes [EC-D: epirubicin (90 mg/m^2) and cyclophosphamide (600 mg/m^2) given every 3 weeks for four cycles followed by docetaxel (100 mg/m^2) every 3 weeks for four cycles] from 2003 to 2010. Patients were staged according to the tumor-node-metastasis (TNM) system. Clinical response was assessed by palpation, breast ultrasound, mammography, and/or magnetic resonance imaging before systemic therapy and before curative surgery. Clinical responses were evaluated according to the response evaluation criteria in solid tumors (RECIST) criteria [28] every two cycles. Patients treated with FEC75 or FAC60 with partial response received surgical treatment after four cycles of chemotherapy and two additional cycles of FEC75 or FAC60 were administered after surgery. Patients treated with EC-D underwent breast surgery after completion of chemotherapy. Patients with positive hormone receptor tumors received radiotherapy and endocrine therapy. The extent of residual disease was measured in the surgical specimen. The primary endpoint for this study was pCR defined as the absence of invasive cancer in the breast and axillary lymph nodes at the time of definitive surgery. The secondary endpoints for the study were disease-free survival (DFS) and overall survival (OS). Patients were followed up according to the breast cancer guidelines.

Tumor Samples and Tissue Microarrays

We analyzed 140 representative formalin-fixed, paraffin-embedded tumor core biopsies obtained before neoadjuvant treatment. Paraffin blocks were stored at room temperature. Samples were identified only by an identification number assigned to each patient. A stained section of each tumor sample was prepared to confirm the diagnosis and to identify representative tumor areas. Tissue microarrays were prepared from formalin-fixed, paraffin-embedded tissue taken from three representative areas of each tumor. Serial 5-μm sections were obtained for immunohistochemical, fluorescence *in situ* hybridization (FISH), and chromogenic *in situ* hybridization (CISH) analyses.

Immunohistochemistry

Prediluted antibodies for estrogen receptor (ER; clone EP1), progesterone receptor (PR; clone 636) and Ki67 (clone MIB-I) were obtained from Dako (Glostrup, Denmark). Sections were processed in a PT Module using Dako high pH buffer (Dako) for deparaffinization and antigen retrieval. Sections for the Ki-67 study were processed with Dako low pH buffer. All immunohistochemical stains were performed in an Autostainer Link using the EnVision method (Dako). *HER2* overexpression was analyzed using the HercepTest assay (Dako). Tumors were classified as ER or PR positive when at least 1% of the tumor cells showed staining in the nuclei cells [29]. *HER2* was considered overexpressed when a uniform intense (3+) membrane staining was present in >30% of invasive tumor cells [30]. The percentage of Ki67-stained nuclei was evaluated independently of the intensity and its positivity cutoff value

was ≥20% [31]. Two pathologists independently evaluated all immunostainings, and discordant results were reviewed to reach an agreement.

In situ *Hybridization Analyses*

HER2 gene status was confirmed for all patients by FISH. *TOP2A* gene status was evaluated by CISH and FISH in equivocal cases. A good correlation of the results for *TOP2A* obtained by FISH or CISH has been previously reported [32]. We used the *HER2* FISH pharmDX (Dako) and the *TOP2A* FISH pharmDX (Dako) assays, respectively. The CISH assay was performed using Dako dual color assay (Dako). All tests were performed following the manufacturer's recommendations. Fluorescence signals were evaluated using an Olympus BX51 fluorescent microscope and appropriate filter sets. The assessment of FISH results for *TOP2A* and *HER2* was performed in ≥60 nuclei per case. *TOP2A* gene status was assessed by CISH in ≥30 tumor cells per case. *HER2* gene amplification was defined when the ratio *HER2*/CEP17 was ≥2 in accordance with the manufacturer's recommendations. *TOP2A* amplification was considered when the ratio *TOP2A*/CEP17 was ≥2 and *TOP2A* deletion was considered when the ratio *TOP2A*/CEP17 was <0.8. We use the term CEP17 duplication throughout the text as defined previously [21]. The cutoff value for CEP17 duplication was determined as >1.86 observed CEP signals per cell [21,26].

Statistical Analyses

DFS was defined as the time from initiation of treatment to date of first relapse (local, regional, contralateral or metastatic), second primary cancer, or death resulting from any cause (whichever occurred first). OS was defined as the time from sample collection to death resulting from any cause. Patients lost to follow-up were censored at the last contact. Quantitative variables between groups were compared using the Student's *t* test, and categorical variables were compared using the Chi-square or Fisher exact test. The univariable association between variables and pCR was assessed using the Chi-square test. The multivariable logistic regression model for pCR was adjusted for age at diagnosis (<50 *vs* ≥50 years), tumor stage (T2 *vs* T3 *vs* T4), nodal stage (negative *vs* positive), histologic grade (1 or 2 *vs* 3), neoadjuvant chemotherapy (FEC75/FAC60 *vs* EC-D), ER (negative *vs* positive), PR (negative *vs* positive), *HER2* (normal *vs* amplified), *TOP2A* status (normal *vs* amplified), and CEP17 status (normal *vs* duplicated). Kaplan-Meier and log-rank analyses were used to compare DFS and OS. The Cox proportional hazard model with a single covariate was used to obtain the hazard ratios (HRs) for relapse or death and associated 95% confidence interval (95% CI). *HER2* (normal *vs* amplified), *TOP2A* status (normal *vs* amplified), and CEP17 status (normal *vs* duplicated) as individual variables were entered in a final Cox model adjusted for traditional prognostic factors including age at diagnosis (<50 *vs* ≥50 years), nodal stage (negative *vs* positive), tumor size (T2 *vs* T3 *vs* T4), histologic grade (1 or 2 *vs* 3), neoadjuvant chemotherapy (FEC75/FAC60 *vs* EC-D), ER (negative *vs* positive), PR (negative *vs* positive), and Ki67 (low *vs* high). The Cox proportional hazard model was used in an exploratory analysis of tumors to analyze tumors with either *TOP2A* amplification or CEP17 duplication; we refer to these tumors throughout the text as tumors with combined *TOP2A* or CEP17 duplication. A two-sided *P* value ≤ .05 was considered significant.

Statistical analyses were performed using the SPSS 19.0 statistical software package (SPSS, Chicago, IL).

Patient selection, assay performance, and data analysis concurred to the reporting recommendations for tumor marker prognostic studies (REMARK) guidelines [33].

Results

Patient Characteristics

Patient and tumor characteristics are summarized in Table 1. A total of 101 of the 140 patients had T3 or T4 tumors (72.1%), and 93 had lymph node–positive tumors (66.4%); these factors explain the observed low rate of conservative treatment (37.1%). A pCR after neoadjuvant chemotherapy was achieved in 13 patients (9.3%). Four patients (7.3%) achieved pCR after FEC75/FAC60 and nine (10.6%) patients after EC-D treatment. No significant differences in pCR were observed according to type of neoadjuvant treatment or tumor stage (Supplementary Table 1).

Association of CEP17 Duplication with Clinicopathologic Variables

Table 2 shows the associations of CEP17 status with patient characteristics and tumor-related variables. Status of *HER2*, *TOP2A*, and CEP17 duplication was assessable in 137 (97.9%) tumors. *HER2* amplification was found in 24 samples (17.5%), *TOP2A* was amplified in 7 (5.1%) of the tumors, *TOP2A* deletion was found in 6 samples (4.4%), and CEP17 duplication was detected in 13 (9.5%) samples. Tumors with combined *TOP2A* amplification and CEP17

Table 1. Basic Patient and Tumor Characteristics.

Variable		*n* (%)
N		140
Age, years	Mean ± SD	53.7 ± 13.5
	Median (range)	51 (25.5-86.1)
Menopausal status	Premenopausal	65 (46.4)
	Postmenopausal	75 (53.6)
Tumor stage	II	50 (35.7)
	III	90 (64.3)
Tumor status	T2	39 (27.9)
	T3	42 (30.0)
	T4	59 (42.1)
Node status	N0	47 (33.6)
	N1	63 (45.0)
	N2	26 (18.6)
	N3	4 (2.9)
Tumor grade	1	11 (7.9)
	2	63 (45.0)
	3	66 (47.1)
ER	Positive	87 (62.1)
	Negative	53 (37.9)
PR	Positive	68 (48.6)
	Negative	72 (51.4)
Ki67 status	<20%	74 (42.9)
	≥20%	60 (52.9)
	Missing data	6 (4.3)
HER2	Normal	113 (81)
	Amplification	24 (17)
	Missing data	3 (2)
TOP2A	Normal	124 (89)
	Amplification	7 (5)
	Deletion	6 (4)
	Missing data	3 (2)
Neoadjuvant therapy	FEC75/FAC60	55 (39.3)
	EC-D	85 (60.7)
Surgical treatment	Mastectomy	88 (62.9)
	Lumpectomy	52 (37.1)
Pathologic response	Complete	13 (9.3%)
	Residual disease	127 (90.7)

Table 2. Correlation between Clinicopathologic Characteristics and CEP17 Status.

Variable		n (%)	CEP17 Status		P*
			Normal	Duplicated	
CEP17		137	124 (90.5)	13 (9.5)	
Age, years	≤50	65 (47.5)	61 (49)	4 (30.8)	.206
	>50	72 (52.5)	63 (51)	9 (69.2)	
Menopausal status	Premenopausal	63 (46)	59 (47)	4 (30.8)	.247
	Postmenopausal	74 (54)	65 (53)	9 (69.2)	
Tumor stage	II	49 (35.8)	44 (33.5)	5 (38.5)	.830
	III	88 (64.2)	80 (64.5)	8 (61.5)	
Tumor status	T2	38 (27.7)	33 (33.5)	5 (38.6)	.606
	T3	42 (30.7)	38 (30)	4 (30.7)	
	T4	57 (41.6)	53 (43)	4 (30.7)	
Node status	N0	46 (33.5)	42 (34)	4 (31)	.538
	N1	62 (45.5)	54 (44)	8 (61.5)	
	N2	25 (18)	24 (19)	1 (7.5)	
	N3	4 (3)	4 (3)	0 (0)	
Tumor grade	1	11 (8)	11 (9)	0 (0)	.499
	2	62 (45)	55 (44)	7 (54)	
	3	64 (47)	58 (47)	6 (46)	
ER	Positive	86 (62.8)	77 (62)	9 (69.2)	.613
	Negative	51 (37.2)	47 (38)	4 (30.8)	
PR	Positive	68 (49.6)	62 (50)	6 (46)	.792
	Negative	69 (50.4)	62 (50)	7 (54)	
Ki67 status	<20%	73 (53.3)	66 (53)	7 (54)	.937
	≥20%	60 (50.4)	54 (43.5)	6 (46)	
	Missing data	4 (2.9)	4 (3)	0 (0)	
HER2	Normal	112 (82)	103 (83)	9 (69)	.166
	Amplification	23 (17)	19 (15)	4 (31)	
	Missing data	2 (1)	2 (2)	0 (0)	
TOP2A	Normal	124 (91)	111 (89.5)	13 (100)	.220
	Amplification	7 (5)	7 (5.5)	0 (0)	
	Deletion	6 (4)	6 (5)	0 (0)	
Neoadjuvant therapy	FEC75/FAC60	84 (61.3)	76 (61)	8 (61.5)	.986
	EC-D	53 (38.7)	48 (39)	5 (38.5)	
Surgical treatment	Mastectomy	86 (62.9)	78 (62.9)	8 (61.5)	.923
	Lumpectomy	51 (37.1)	46 (37.1)	5 (38.5)	
Pathologic response	Complete	12 (8.7)	9 (7.3)	3 (23)	.055
	Residual disease	125 (91.3)	115 (92.7)	10 (77)	

* P values were calculated using the Chi-square test.

duplication alterations were present in 20 (14.6%) of the tumors. CEP17 duplication was not associated with *HER2* gene amplification (P = .166), high grade (P = .499), ER negativity (P = .613), or a high Ki67 (P = .937). Tumors with *HER2* amplification had *TOP2A* amplification in three cases (42.9%, P = .062), *TOP2A* deletion in three cases (50%, P = .028), and CEP17 aneusomy in two cases (40% P = .164). *HER2* amplifications were significantly associated with *TOP2A* alterations (amplifications and deletions; P = .003) and with CEP17 aneusomy (P = .048; data not shown).

Table 3. Multivariable Analysis of Predictive Factors for pCR.

Variable	Score	pCR	
		OR (95% CI)	P*
ER	Positive	1	
	Negative	7.12 (1.69-30.03)	.007
TOP2A	Normal	1	
	Amplification	6.97 (0.96-50.12)	.054
CEP17	Normal	1	
	Duplication	6.55 (1.25-34.29)	.026
HER2	Normal	1	
	Amplification	0.804 (0.18-3.597)	.755
TOP2A/CEP17 [†]	Normal	1	
	Altered	6.71 (1.66-27.01)	.007

* Logistic regression models adjusted for known prognostic factors (age, nodal status, tumor size, tumor grade, and hormone receptor status) and neoadjuvant therapy.

[†] Tumors with combined *TOP2A* amplification and CEP17 duplication.

Association of Clinicopathologic Parameters with pCR

In the multivariable analysis, CEP17 duplication associated with a high percentage of pCR [odds ratio (OR) 6.55, 95% CI 1.25-34.29, P = .026] and *TOP2A* gene amplification showed a benefit with borderline significance (OR 6.97, 95% CI 0.96-50.12, P = .054; Table 3). When *TOP2A* amplifications and CEP17 duplication were combined, we observed a significant association with pCR (OR 6.71, 95% CI 1.66-27.01, P = .007; Table 3). We did not find a significant difference in the benefit of treatment with anthracyclines when assessing deletion (P = .429) or combined *TOP2A* amplifications and deletions (P = .089) in univariable analysis. *HER2* amplifications showed no direct association with pCR (P = .144). When pCR was assessed according to treatment received (Supplementary Table 1), CEP17 duplications were the only factor associated with better response to FEC70/FAC65 in both univariable (P = .004) and multivariable analyses (OR 14, 95% CI 1.427-137.324, P = .025). Meanwhile, the multivariable analysis showed that ER status (OR 12.68, 95% CI 1.427-137.324, P = .011) and TOP2A/CEP17 (OR 6.31, 95% CI 1.981-81.186, P = .007) were associated with better response to EC-D (data not shown).

Prognostic Associations with DFS and OS

The univariable analysis showed that CEP17 duplication, *HER2* amplification, and *TOP2A* amplification were not significantly associated with DFS or OS (Table 4). However, when *TOP2A* amplification and CEP17 duplication were combined, Kaplan-Meier and log-rank analyses showed a significant association with better DFS (P = .026) and a borderline non-significant trend with OS (P = .054; Figure 1). The univariable analysis showed a similar association of the combination of *TOP2A* amplification and CEP17 duplication with DFS (HR 4.34, 95% CI 1.05-17.92, P = .042) and OS (HR 3.70, 95% CI 0.89-15.34, P = .071; Table 4). However, after adjustment for the classic prognostic factors, age, and neoadjuvant therapy, the multivariable analysis showed that CEP17 duplication, TOP2A amplification, HER2 amplification, and the combination of *TOP2A* amplifications and CEP17 duplication failed to show an association with DFS or OS (Table 5).

Discussion

The main finding in this study is that CEP17 duplication associates with pCR to anthracycline-based neoadjuvant therapy in patients with breast cancer. To our knowledge, this is the first study to analyze the association of CEP17 duplication with response to neoadjuvant

Table 4. Biomarker Analysis and Relation with DFS and OS.

Variable	Score	n	DFS		OS	
			HR (95% CI)	P	HR (95% CI)	P*
CEP17	Normal	124	1		1	
	Duplicated	13	2.64 (0.64-10.9)	.179	2.42 (0.58-10.02)	.223
HER2	Normal	113	1		1	
	Amplified	24	0.97 (0.45-2.08)	.960	0.96 (0.44-2.06)	.907
TOP2A	Non-amplified	130	1		1	
	Amplified	7	21.9 (0.92-5275)	.269	21.73 (0.04-11545)	.336
Ki67	≥20%	60	1		1	
	<20%	74	1.89 (1.04-3.46)	.036	1.91 (1.02-3.58)	.044
TOP2A/CEP17 [†]	Normal	117	1		1	
	Altered	20	4.35 (1.05-17.93)	.042	3.71 (0.89-15.34)	.071

* Univariable Cox proportional hazard models based on 136 patients, 44 recurrences, and 40 deaths.

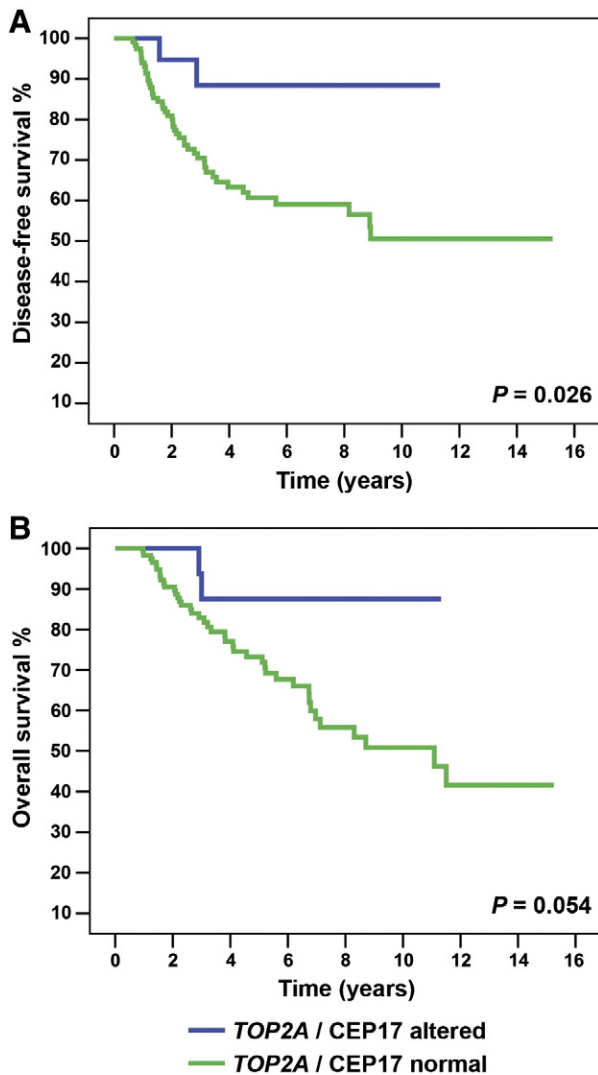[†] Tumors with combined *TOP2A* amplification and CEP17 duplication.

Figure 1. Association of combined *TOP2A* amplification and CEP17 duplication with patient outcome. DFS (A) and OS (B) Kaplan-Meier curves and log-rank tests according to the presence or absence of tumors with combined *TOP2A* amplification and CEP17 duplication.

chemotherapy using pCR as a surrogate marker of chemosensitivity. Our rationale in investigating the association of CEP17 duplication and response to chemotherapy was based on the recognition that

Table 5. Multivariable Analyses for All Prognosis Variables and Relation with DFS and OS.

Variables	DFS		OS	
	HR (95% CI)	*P*	HR (95% CI)	*P**
Age (<50 *vs* ≥50 years)	1.03 (0.5-2.15)	.91	1.09 (0.5-2.41)	.81
Lymph nodes (negative *vs* positive)	2.95 (1.23-7.07)	.01	3.12 (1.21-8.03)	.01
Tumor size (T2 *vs* T3 *vs* T4)	1.78 (0.85-3.7)	.02	1.63 (0.68-3.91)	.9
Tumor grade (1 *vs* 2 *vs* 3)	1.53 (0.77-3.03)	.22	2.07 (1.06-4.05)	.03
ER (positive *vs* negative)	2.20 (1.24-4.01)	.01	1.95 (0.93-4.07)	.07
pCR (positive *vs* negative)	4.41 (0.54-30.65)	.08	4.49 (0.58-34.72)	.15
Ki67 (<20% *vs* ≥20%)	1.29 (0.65-2.54)	.45	1.33 (0.65-2.71)	.43
Treatment (EC-D *vs* FEC/FAC)	1.17 (0.61-2.24)	.62	1.07 (0.54-2.12)	.83
CEP17 (duplicated *vs* normal)	2.46 (0.54-11.06)	.24	2.29 (0.51-10.35)	.27
HER2 (amplified *vs* normal)	1.23 (0.51-2.97)	.63	1.06 (0.43-2.62)	.89
TOP2A/CEP17 † (altered *vs* normal)	3.91 (0.89-17.2)	.71	3.72 (0.84-16.43)	.08

 * Multivariable Cox proportional analysis based on 136 patients, 44 recurrences, and 40 deaths.

 † Tumors with combined *TOP2A* amplification and CEP17 duplication.

CEP17 duplication could be a potential marker of genomic instability and DNA repair dysfunction [26,34]. Chromosome 17 is the second highest density gene in the human genome, and it includes several key genes that have a role in breast cancer (*HER2*, *TOP2A*, *BRCA1*, and *Tau*) and DNA repair (*P53*, *RAD51C*, and *RAD52B*) [35,36]. Analyses of rearrangement have shown that chromosome 17 harbors a region of high genomic instability centered around 17q12 and characterized by multiple gene amplifications and deletions [37–39]. This pattern has traditionally been associated with polysomies of chromosome 17. However, recent data using array comparative genomic hybridization suggest that chromosome 17 polysomies detected by FISH or CISH are in fact very rare in breast cancer; what is being detected are gains or amplification in the centromere region of chromosome 17 rather than a copy number gain of the whole chromosome 17 [24,25]. An increase in the copy numbers of CEP17 might cause several key genetic changes, such as *HER2* amplification, *TOP2A* amplification or deletion, *P53* loss, and *BRCA1* loss. All these chromosome abnormalities have been linked to mechanisms of breast tumorigenesis including cell proliferation, tumor angiogenesis, and reduced apoptosis [38,39]. Together, these findings suggest that CEP17 duplication is an indicator of genetic instability around 17q12 locus that could be linked to aberrations in the copy number of DNA repair genes, such as *TOP2A*. Therefore, analysis of CEP17 duplication may allow for an improvement of treatment efficacy based on therapies targeting DNA repair genes.

Our results showed that CEP17 duplication was present in 9.3% of the tumors, a result that is somehow inferior to results reported by others. However, the interlaboratory reproducibility of CEP17 testing is unknown, and the prevalence differs among studies, ranging from 8% to 68% depending on the type of material examined, cell counting methods, and the threshold criteria for CEP17 duplication [36]. Moreover, there are conflicting results in earlier studies in relation to the correlation between CEP17 duplication and a more aggressive tumor phenotype [36]. Our results did not show an association between CEP17 duplication and high tumor grade, lymph node metastasis, ER negativity, *HER2* amplification, or high Ki67.

Another finding of interest in our study is that *TOP2A* gene amplification correlated with pCR to anthracycline-based neoadjuvant therapy. However, the response was of borderline significance (OR 6.97, 95% CI 0.96-50.12, *P* = .054), but when combined with CEP17 duplication, the pCR response was significant (OR 6.71, 95% CI 1.66-27.01, *P* = .007). The small number of patients in our study might explain that *TOP2A* gene amplification alone (found in only 5% of tumors) was not associated with pCR. These observations suggest that patients with tumors that harbor gene alterations that may increase *TOP2A* copy number benefit more from anthracycline-based neoadjuvant therapy. We hypothesized that anthracycline sensitivity is not only the result of an increased number of genes secondary to gene amplification of *TOP2A* but also the result of concomitant gains in pericentromeric regions on chromosome 17 (CEP17 duplication). Thus, CEP17 duplication would be an indirect mechanism of increasing *TOP2A* gene copy number. However, we cannot yet identify which of these chromosomal abnormalities might be associated with the underlying mechanism of chemosensitivity.

As in previous studies [40,41], we found that *HER2* amplification failed to have a predictive value, supporting that patients with *HER2*-negative tumors may benefit from treatment with

anthracyclines. We also observed that patients with *TOP2A* normal tumors might have some additional benefit from treatment with anthracyclines. Our results suggest that other mechanisms, such as the immune response, tumor-stromal interactions, and CEP17 duplication, may participate in the increased sensitivity to anthracyclines in these patients [42–45]. Therefore, the use of anthracyclines should not be limited to patients with *HER2* amplification or *TOP2A* amplification.

We were unable to identify any statistically significant relationship between CEP17 duplication and patient outcome. Our results are consistent with two previous studies [21,27] that analyzed prognostic and predictive associations of CEP17 duplication in early-stage breast cancer patients treated with adjuvant chemotherapy regimens containing anthracyclines and non-anthracyclines. Bartlett et al. [21] assessed the role of CEP17 duplication in 1931 tumors from 2391 women enrolled onto the UK National Epirubicin Adjuvant Trial (NEAT/BR9601). Interestingly, although this study also failed to find a significant association of CEP17 duplication with patient prognosis (DFS HR 1.12, 95% CI 0.92-1.36, P = .24, OS HR 1.17, 95% CI 0.95-1.44, P = .13), it showed that the benefit on survival from the addition of epirubicin was confined to tumors with CEP17 duplication. Similar results were obtained by Pritchard et al. [27] in the randomized controlled mammary 5 (MA.5) adjuvant trial. The results did not identify a significant association of CEP17 with risk of recurrence (HR 1.15, 95% CI 0.86-1.54, P = .34) or risk of death (HR 1.39, 95% CI 0.90-2.15, P = .14) in multivariable analysis. However, in patients whose tumors exhibit CEP17 duplication and who received anthracyclines, there was a significant benefit for recurrence-free survival (HR 0.59, 95% CI 0.37-0.92, P = .02) and OS (HR 0.61, 95% CI 0.37-1.01, P = .05) in univariable analysis. This association disappeared after adjustment for other variables.

The potential limitations of our study are the restricted sample size and the retrospective design, which could bias patient selection. In addition, the absence of a study group receiving non-anthracycline-based chemotherapy meant we were unable to distinguish whether the pCR rate was attributed to higher sensitivity to anthracyclines or to chemotherapy with anthracyclines, taxanes, cyclophosphamide, and/or 5-fluorouracil. These results are therefore preliminary and require confirmation in larger prospective studies specifically designed to validate the predictive role of these biomarkers and to compare regimens with and without anthracyclines.

In conclusion, our results suggest that CEP17 duplication may be a useful biomarker of sensitivity to neoadjuvant anthracycline-based chemotherapy. A better understanding of CEP17 abnormalities might help to select patients who benefit most from anthracycline-based chemotherapy regimens and to identify patients in whom toxicity from these drugs could be avoided.

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