1. Introduction

Epirubicin, which belongs to the anthracycline family, is one of the most aggressive drugs against breast cancer and epirubicin-based regimens such as 5-FU plus epirubicin pulse cyclophosphamide (FEC) and epirubicin plus cyclophosphamide (EC) are widely used in adjuvant and neoadjuvant as well as in metastatic settings. These epirubicin-based regimes, however, although very active, are not necessarily effective for all patients. In fact, response rates of metastatic breast cancers to epirubucin-based regimens reportedly range from 50% to 60% [1,2]. On the other hand, adverse events such as leucopenia and alopecia are observed in virtually all patients treated with these regimens although their severity differs from patient to patient. In addition, a small but significant proportion of patients develop serious adverse events such as cardiac failure and myeloproliferative diseases. In order to increase the efficiency of chemotherapy and avoid unnecessary adverse events, it is therefore very important to administer chemotherapy to those patients who are likely to respond and not to those who are unlikely to respond. For purpose, reliable predictive factors for response to chemotherapy need to be developed. Until now, various biological parameters, including HER-2 [3], p-glycoprotein [4], p53 [5], estrogen receptor (ER) [6], S-phase fraction [7], Ki-67 [7], have been proposed as candidate predictive factors for response to epirubicin-based and doxorubicin-based regimens (doxorubicin is another anthracycline) but their clinical value remains controversial so that they have not yet been integrated in daily practice.

Among the predictive factors so far studied, *HER-2* gene amplification and HER-2 overexpression have been attracting a great deal of attention, and a significant association between *HER-2* gene amplification or HER-2 overexpression and a favorable response to epirubicin-based regimens has been reported [3,8,9]. However, recent studies have shown that such an association between response and HER-2 is indirect and that the direct association occurs between response and the expression of topoisomerase IIalpha (TOP2A), which is a target molecule of epirubicin [10,11]. The *TOP2A* gene is localized close to the *HER-2* gene and is often coamplified with the *HER-2* gene [12]. TOP2A plays a pivotal role in DNA replication and catalyzes the transport of one DNA double helix through another by the transient introduction of DNA double-strand breaks [13]. Anthracyclines including epirubicin and doxorubicin bind to TOP2A and stabilize the DNA double-strand breaks, resulting in cell cycle arrest and apoptosis [13,14]. In fact, an in vitro study has shown that breast cancer cells with TOP2A overexpression are more sensitive to doxorubicin [12]. It has also been reported that TOP2A expression is observed in 20–62% [11,15–19] and TOP2A gene amplification in 12–24% of human breast cancers [11,16,18,20,21]. Several lines of evidence have suggested that anti-tumor activity of the epirubicin-based regimens is associated with TOP2A expression or *TOP2A* gene amplification, although the contradictory results have also been reported [21–24].

In addition to TOP2A, BRCA1 has recently been gaining attention as a predictive factor for response to epirubicin-based regimens. BRCA1 plays an important role in double-strand DNA repair [25], and because epirubicin induces DNA double-strand breaks, it is possible that BRCA1 may modulate the response to epirubicin. In this connection, it has been reported that a mouse cell line deficient in BRCA1 displayed an increased sensitivity to the agents, including doxorubicin, which cause double-strand DNA breaks, and that induction of wild-type BRCA1 resulted in a reduced level of apoptotic cell death after treatment with DNA-damaging agents [26]. It has also been found that overexpression of BRCA1 in murine ovarian cancer cells increased the resistance to doxorubicin [27]. Furthermore, Delaloge et al. reported that 53% of locally advanced breast cancers carrying a BRCA1 mutation showed complete response to the anthracycline-based regimens while only 14% of sporadic breast cancers did, indicating that breast cancers lacking a BRCA1 function due to its mutation are more sensitive to anthracycline-based regimens [28]. Although BRCA1 mutation is rare, a significant proportion of sporadic breast cancers lack BRCA1 expression due to hypermethylation of the promoter region of the *BRCA1* gene [29], overexpression of HMGA1 [30], or overexpression of ID4 [31]. Thus, it is possible that BRCA1 expression may influence the sensitivity of sporadic breast cancers to epirubicin-based regimens. However, this possibility has hardly been investigated.

As mentioned earlier, it has been speculated that TOP2A and BRCA1 may be associated with sensitivity to epirubicin-based regimens, and thus are potentially useful as predictive factors for these regimens. Nevertheless, the association between

BRCA1 and response to epirubicin-based regimens in sporadic breast cancers has yet to be reported. This prompted us to immunohistochemically investigate TOP2A and BRCA1 expression simultaneously in breast cancer tissues obtained before the administration of epirubicin-based regimens (preoperative setting), and to study the relationship between the expression of these two markers and pathological response.

2. Materials and methods

2.1. Patients and tumor samples

For this study, 108 primary breast cancer patients at stage II ($n = 73$), III ($n = 22$), and IV ($n = 13$) were consecutively recruited. They were treated with epribucin-based regimens in the preoperative setting during the period between September 1999 and April 2004 at Osaka University Hospital, Osaka Medical Center for Cancer and Cardiovascular Diseases, and Kyushu University Hospital. Treatment with EC was used for 97 and with FEC for 11 patients and all of them were subsequently treated with breast conserving surgery or mastectomy. The epirubicin-based regimens were administered every 3 weeks for 3–6 cycles (3 cycles for 47 patients, 4 cycles for 45 patients, 5 cycles for one patient, and 6 cycles for seven patients). The remaining eight patients were treated with only 2 cycles of EC ($n = 5$) or FEC ($n = 3$) because of disease progression, and were switched to other chemotherapy (paclitaxel or docetaxel) before surgery. The dose of epirubicin for both the EC and FEC regimens was 60 mg/m^2 epirubicin for 107 patients and 100 mg/m^2 for one patient. Tumor tissue samples were obtained from primary tumors by means of vacuum-assisted core needle biopsy prior to preoperative chemotherapy. The samples were subjected to pathological diagnosis for determination of ER, PR, and HER-2 status as well as immunohistochemical study of TOP2A and BRCA1. This study was approved by the IRB of Osaka University Graduate School of Medicine.

2.2. Assessment of tumor grade and pathological response

Nuclear grade, mitotic score, and tubular formation were determined according to the criteria specified by Elston and Ellis [32]. Since the association between pathological response and patient prognosis is much stronger than that between clinical response and patient prognosis [33–35], we adopted pathological response, but not clinical response, to evaluate the effect of epirubicin-based regimens in the present study. Pathological response of breast tumors was evaluated in 100 patients who were treated with three or more cycles of the epirubicin-based regimens alone. Multiple slides prepared from primary breast tumors after preoperative chemotherapy were examined and chemotherapeutic effect was determined as for the breast tumors according to the criteria specified in the General Rules for Clinical and Pathological Recording of Breast Cancer 2005 [36]. These criteria define Grade 0 as no response (almost no change in cancer cells), Grade 1 as slight response (1a: mild changes in cancer cells regardless of the area; 1b: marked changes in one-third or more but less than two-thirds of tumor cells), Grade 2 as marked response (marked changes in two-thirds or more of tumor cells), and Grade 3 as complete response (necrosis or disappearance of all tumor cells). The eight patients who showed a progressive disease after 2 cycles of the epirubicin-based regimens and were switched to other types of chemotherapy were classified as pathological non-responders.

2.3. Immunohistochemistry of HER-2, TOP2A, and BRCA1 expression

The expression of HER-2, TOP2A, and BRCA1 was evaluated immunohistochemically by using the tumor specimens obtained as described under patients and tumor samples. Sections prepared from the formalin-fixed paraffin-embedded tumor specimens were deparaffinised and rehydrated in graded alcohol. Antigens were retrieved by incubating the sections in 10 mmol/l citrate buffer (pH 6.0) at 95 °C for 50 min for TOP2A or by boiling for 15 min in a microwave oven for BRCA1. After quenching endogenous peroxidase with 3% H_2O_2 in methanol for 20 min, the resultant slides were treated with Block Ace (Dainippon Sumitomo Pharmaceutical, Osaka, Japan) for 30 min at room temperature. The samples were then incubated overnight at 4 °C with a polyclonal rabbit anti-c-erbB2 antibody (1:100 dilution; Nichirei Biosciences Inc., Tokyo, Japan) for HER-2, with a mouse monoclonal anti-TOPOIIα antibody (1:70 dilution; KiS1, DakoCytomation Inc., Carpinteria, CA) for TOP2A, or with a mouse monoclonal anti-BRCA1 antibody (1:70 dilution; Ab-1, Oncogene Science, Cambridge, MA) for BRCA1. They were subsequently incubated at room temperature for 30 min with the ABC Kit (Vector Laboratories, Burlingame, CA) using biotinylated anti-rabbit immunoglobulin G antibody for HER-2 or biotinylated anti-mouse immunoglobulin G (IgG) antibody for BRCA1. For TOP2A, incubation was performed with EnVision+ System Peroxidase (DakoCytomation) according to the manufacturer's instructions. Finally, the antibody complex was visualized with 3,3′-diaminobenzidine tetrahydrochloride (Merck, Darmstadt, Germany) and the sections were counter-stained with hematoxylin.

Positive reactions for HER-2 were scored as four grades, as previously reported [37], according to the intensity and pattern of the staining. The four grades were: 0

(no or less than 10% membrane staining in tumor cells); 1+ (faint membrane staining in more than 10% of tumor cells, partial staining of the membrane); 2+ (weak-to-moderate but complete membrane staining in more than 10% of tumor cells); 3+ (strong and complete membrane staining in more than 10% of tumor cells) Grade 2+ and 3+ tumors were considered to be HER-2 positive. The most actively stained lesions were selected microscopically and nuclear staining was counted in 1000 cancer cells without knowledge of patients outcome, and 5% and 10% were used as the respective cut-off values for TOP2A and BRCA1 according to the method described previously [17,38].

2.4. ER and PR assay

ER and progesterone receptor (PR) protein levels in the tumor specimens obtained before preoperative chemotherapy were determined in 83 cases with immunohistochemistry (cut-off value was 10% for both ER and PR) or in 21 cases with an enzyme immunoassay using kit from Abbott Research Laboratories (Chicago, IL) according to the manufacturer's instructions (cut-off values for ER and PR were 13 and 10 fmol/mg, respectively).

2.5. Statistical methods

The relationship between clinicopathological or biological parameters and pathological response was evaluated with the Fisher's exact test. Multivariate analysis of the relationship of TOP2A and BRCA1 expression with pCR was determined using a logistic regression method to obtain the odds ratio and 95% confidence interval, being adjusted for menopausal status, tumor size, lymph node metastasis, distant metastasis, nuclear grade, ER, PR, and HER-2 status. Statistical significance was assumed for $P < 0.05$.

3. Results

3.1. Relationship between clinicopathological or biological parameters and pathological response to epirubicin-based regimens

Pathological response was divided into two categories, i.e., pathological complete response (pCR, Grade 3) and non-pCR (Grades 0, 1a, 1b, and 2) for evaluation of its relationship with clinicopathological parameters (Table 1). The pCR rate (13%) of small tumors (≤5 cm) was significantly ($P < 0.05$) higher than that (0%) of large tumors (>5 cm). No statistically significant association was observed between pCR rate and menopausal status, lymph node status, distant disease status, nuclear grade, mitotic score, or tubular formation.

Table 1
Relationship between clinicopathological factors and pathological response to epirubicin-based regimens

Pathological response[a]	Non-pCR	pCR	*P*-value
Menopausal status			
Pre-	62 (94)[b]	4 (6)	0.30
Post-	37 (88)	5 (12)	
Tumor size			
≤5 cm	58 (87)	9 (13)	<0.05
>5 cm	41 (100)	0 (0)	
Lymph node metastasis			
Negative	31 (91)	3 (9)	0.99
Positive	68 (92)	6 (8)	
Distant metastases			
Negative	86 (91)	9 (9)	0.59
Positive	13 (100)	0 (0)	
Nuclear grade			
I + II	45 (94)	3 (6)	0.71
III	45 (90)	5 (10)	
Unknown	9 (90)	1 (10)	
Mitotic score			
I + II	56 (95)	3 (5)	0.25
III	34 (87)	5 (13)	
Unknown	9 (90)	1 (10)	
Tubular formation			
I + II	19 (90)	2 (10)	0.67
III	71 (92)	6 (8)	
Unknown	9 (90)	1 (10)	

[a] Pathological response was classified as described in Section 2.
[b] % of patients.

The pathological response was further studied in terms of its relationship with biological parameters including ER, PR, and HER-2, but no significant association with any of these parameters was detected (Table 2).

Table 2
Relationship between biological parameters and pathological response to epirubicin-based regimens

Pathological response[a]	Non-pCR	pCR	*P*-value
Estrogen receptor			
Positive	28 (90)[b]	3 (10)	0.99
Negative	67 (92)	6 (8)	
Unknown	4 (100)	0 (0)	
Progesterone receptor			
Positive	28 (90)	3 (10)	0.99
Negative	51 (89)	6 (11)	
Unknown	20 (100)	0 (0)	
HER-2 status			
Positive	24 (89)	3 (11)	0.43
Negative	70 (93)	5 (7)	
Unknown	5 (83)	1 (17)	

[a] Pathological response was classified as described in Section 2.
[b] % of patients.

3.2. TOP2A and BRCA1 expression and their relationship with clinicopathological and biological parameters or pathological response

Expression of TOP2A and BRCA1 was examined immunohistochemically in 108 tumor samples obtained before preoperative chemotherapy. Representative immunohistochemical results are shown in Fig. 1. Tumors with a high mitotic score (III) were significantly more likely to show a higher TOP2A positivity than tumors with a low mitotic score (I + II) (67% vs. 29%, $P < 0.001$). Tumors with positive HER-2 were significantly more likely to show a higher TOP2A positivity than those with negative HER-2 (59% vs. 35%, $P < 0.05$) (Table 3). Tumors with

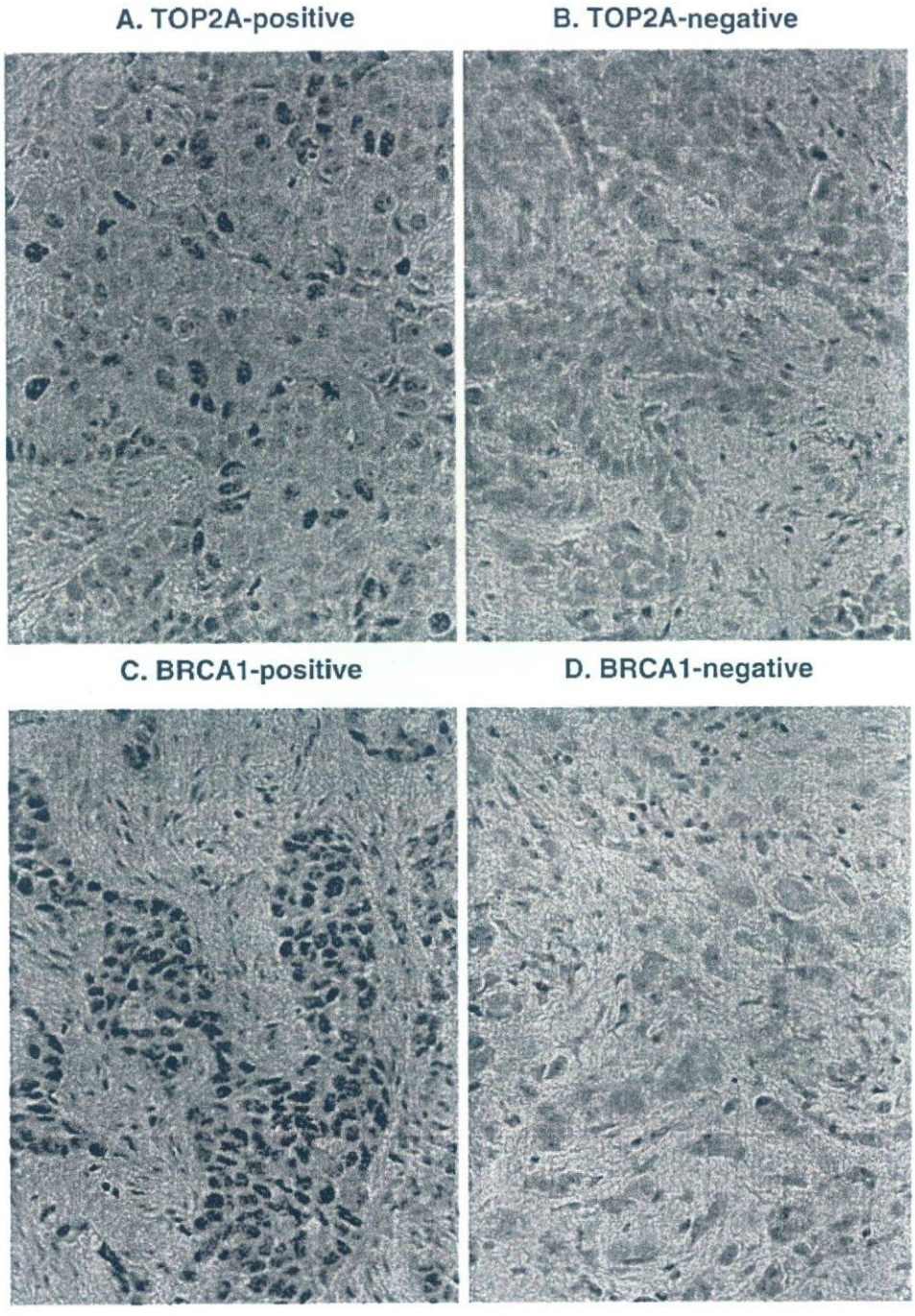

Fig. 1. Immunohistochemical staining of TOP2A and BRCA1. Representative results of immunohistochemical staining of TOP2A and BRCA1 (400×). Nuclear staining of TOP2A-positive (A), TOP2A-negative (B), BRCA1-positive (C), and BRCA1-negative (D) is seen in tumor cells.

Table 3
Relationship between TOP2A or BRCA1 positivity and clinicopathological factors

	TOP2A positivity (%)	*P*-value	BRCA1 positivity (%)	*P*-value
Menopausal status				
Pre-	36	0.11	55	0.43
Post-	52		45	
Tumor size				
≤5 cm	43	0.99	52	0.84
>5 cm	41		49	
Lymph node metastasis				
Negative	47	0.53	53	0.83
Positive	41		50	
Nuclear grade				
I + II	35	0.10	63	0.07
III	52		44	
Mitotic score				
I + II	29	<0.001	54	0.83
III	67		51	
Tubular formation				
I + II	52	0.45	62	0.46
III	42		51	
Estrogen receptor				
Positive	29	0.08	52	0.99
Negative	49		51	
Progesterone receptor				
Positive	32	0.07	48	0.82
Negative	53		46	
HER-2 status				
Positive	59	<0.05	52	0.99
Negative	35		51	

negative ER and those with negative PR were also more likely, but not significantly so, to show a higher TOP2A positivity than those with, respectively, positive ER (49% vs. 29%, $P = 0.08$) or positive PR (53% vs. 32%, $P = 0.07$) (Table 3). With respect to BRCA1, tumors with a low nuclear grade (I + II) were more likely to show a higher BRCA1 positivity than those with a high nuclear grade (III) (63% vs. 44%, $P = 0.07$).

The relationship between TOP2A or BRCA1 expression and pathological response is shown in Table 4. The pCR rate for TOP2A-positive tumors (17%) was significantly ($P < 0.005$) higher than that for TOP2A-negative tumors (2%). The pCR rate for BRCA1-negative tumors (11%) was higher than that for BRCA1-positive tumors (5%) but the difference was statistically not significant ($P = 0.31$). Multivariate analysis of TOP2A and BRCA1 expression adjusted for menopausal status, tumor size, lymph node metastasis, distant metastasis, nuclear grade, ER, PR, and HER-2 status showed that TOP2A expression was a significant factor which associated with pCR,

Table 4
Relationship between TOP2A or BRCA1 expression and pathological response to epirubicin-based regimens

Pathological response[a]	Non-pCR	pCR	*P*-value
TOP2A			
Positive	38 (83)[b]	8 (17)	<0.005
Negative	61 (98)	1 (2)	
BRCA1			
Positive	52 (95)	3 (5)	0.31
Negative	47 (89)	6 (11)	

[a] Pathological response was classified as described in Section 2.
[b] % of patients.

Table 5
Multivariate analysis of TOP2A and BRCA1 expression with pathological response to epirubicin-based regimens

	Non-pCR[a]	pCR	OR[b] (95% CI[c])	*P*-value
TOP2A				
Negative	61	1	1.00	
Positive	38	8	20.1 (1.44–279)	0.02
BRCA1				
Negative	47	6	1.00	
Positive	52	3	0.44 (0.06–3.15)	0.41

[a] Pathological response was classified as described in Section 2.
[b] Odds ratio adjusted for menopausal status, tumor size, lymph node metastasis, distant metastasis, nuclear grade, ER, PR, and HER2 status.
[c] Confidence interval.

being independent of the other factors (Table 5). Results of the combined analysis of TOP2A and BRCA1 expression are shown in Table 6. The pCR rate for TOP2A-positive and BRCA1-negative tumors (30%) was marginally significantly higher than the rates for TOP2A-positive and BRCA1-positive tumors (8%, $P = 0.06$), and significantly higher than TOP2A-negative and BRCA1-positive tumors (3%, $P < 0.05$), or TOP2A-negative and BRCA1-negative tumors (0%, $P < 0.005$).

Table 6
Relationship between combined TOP2A and BRCA1 expression and pathological response to epirubicin-based regimens

TOP2A	BRCA1	Pathological response[a]		*P*-value
		Non-pCR	pCR	
Positive	Negative	14 (70)[b]	6 (30)	
Positive	Positive	24 (92)	2 (8)	0.06[c]
Negative	Positive	28 (97)	1 (3)	<0.05[c]
Negative	Negative	33 (100)	0 (0)	<0.005[c]

[a] Pathological response was classified as described in Section 2.
[b] % of patients.
[c] *P*-values represent comparison with TOP2A-positive and BRCA1-negative tumors.

4. Discussion

Since TOP2A is a target molecule of epirubicin [14], it has been speculated that TOP2A-positive tumors are more sensitive than TOP2A-negative tumors to epirubicin-based regimens. In this connection, *in vitro* studies using various human cancer cell lines have demonstrated that TOP2A-positive cells are indeed more sensitive to doxorubicin than are TOP2A-negative cells [12]. In addition, some studies have been reported with results that demonstrate a significant association between TOP2A expression and clinical response to epirubicn-based regimens in the neoadjuvant setting [11,16]. However, the relationship between TOP2A expression and pathological response has rarely been investigated [39]. pCR appears to be a better marker than clinical response for the evaluation of sensitivity of breast tumors to chemotherapy because pCR is more closely associated with favorable prognosis than is clinical response [33–35]. For our study, we therefore adopted pCR as an endpoint marker for evaluating the response to epirubicin-based regimens. We were able to show a significantly higher pCR rate (17%) for TOP2A-positive tumors than TOP2A-negative tumors (2% pCR), which is consistent with previously reported findings indicating a significant association between TOP2A expression and clinical response [11,16].

Recently, the importance of TOP2A as a predictive factor for epirubicin-based regimens has also been demonstrated in the adjuvant setting. Knoop et al. reported that patients with *TOP2A* gene amplification show an enhanced recurrence-free survival when treated with CEF than they do when treated with cyclophosphamide plus methotrexate plus 5-fluorouracil (CMF), but a similar increase in recurrence-free survival is not seen in patients with a normal *TOP2A* gene [21]. A similar finding has been reported by Tanner et al., who detected a better relapse-free survival for patients with *TOP2A* gene amplification and treated with tailored and dose-escalated FEC than for those treated with low-dose FEC followed by cyclophosphamide plus thiotepa plus carboplatin (CTCb). This difference was not observed in patients with a normal *TOP2A* gene [23]. These studies further support the notion that TOP2A can serve as a predictive marker of sensitivity to epirubicin-based regimens. Both immunohistochemically determined TOP2A protein expression and *TOP2A* gene amplification have reported to be associated with response to epirubicin-based regimens [21,40,41]. Cardoso et al. conducted a comparative analysis of whether TOP2A expression determined by immunohistochemistry or *TOP2A* gene amplification determined by FISH is more closely associated with response the epirubicin-based regimens, found a stronger association for TOP2A expression [11]. It is further reported that the association between TOP2A overexpression and *TOP2A* gene amplification is not so strong since only 33% of breast tumors with this amplification show TOP2A overexpression, unlike the strong association between HER-2 overexpression and *HER-2* gene amplification [16].

Consistent with previously reported findings [20,42], we found that TOP2A positivity is significantly higher in tumors with a mitotic score of III (67%) or that are ER-negative (49%) or HER-2-positive (59%). Since TOP2A is a key enzyme during cell division and most strongly expressed in the S and G2/M phases [43], TOP2A-positive tumors are thought to have a higher rate of proliferation and a higher proportion of cells in the S or G2/M phases than do TOP2A-negative tumors. It thus seems reasonable to assume that TOP2A-positive tumors are more likely to have a mitotic score of III or to be ER-negative because both types of tumors are highly proliferative. Although HER-2 expression was found to be significantly associated with TOP2A expression, no significant relationship between HER-2 expression and pathological response was observed. In the present study, both Grade 2+ and 3+ were considered to be HER-2 positive but even though HER-2 positive was limited to Grade 3+, we failed to show a significant association of HER-2 status with pathological response (data not shown), indicating that TOP2A rather than HER-2 is a better predictive factor for response to epirubicin-based regimens. Similar results have also been reported [11]. The previously reported association between HER-2 expression and sensitivity to anthracycline-based regimens [3] is thus probably an indirect association mediated through TOP2A.

In addition to the clinical significance of TOP2A, we first investigated that of BRCA1 expression for the prediction of response to epirubicin-based regimens in breast cancers. Although BRCA1 expression alone was not significantly associated with pCR rate, combined analysis of TOP2A and BRCA1 expression was found to be very useful for the prediction of pathological response, i.e., TOP2A-positive and BRCA1-negative tumors showed a pCR rate as high as 30% while other

tumors showed a very low pCR rate of 8% or less. These results seem to suggest that, in addition to TOP2A, BRCA1 modulates sensitivity to epirubicin-based regimens. The exact reason why a lack of BRCA1 expression confers resistance to epirubicin-based regimens is currently unknown but we speculate that DNA double-strand breaks are less likely to be repaired in tumor cells defective in BRCA1 expression, resulting in cell cycle arrest and apoptosis.

In conclusion, we were able to demonstrate that a TOP2A-positive and BRCA1-negative phenotype is predictive of a high sensitivity to epirubicin-based regimens, with a pCR rate of up to 30%. Combined determination of TOP2A and BRCA1 expression by means of immunohistochemistry may be clinically useful for the prediction of tumor response to epirubicin-based regimens. Although TOP2A-positive and BRCA1-negative tumors are generally considered to have a biologically aggressive phenotype leading to a high recurrence rate, our finding seems to suggest that prognosis for such breast tumors, if properly treated with eprubicin-based regimens, could be significantly improved. The dose of epirubicin in the present study appears to be lower than that of a current standard (75 or 100 mg/m^2). However, we believe, even in such a lower dose, it is possible to study the association of biomarkers and response to epirubicin-based regimes. But it is possible that higher doses of epirubicin would give the different results though the essential findings are thought not to be affected so much. Our findings, therefore, need to be confirmed by a future study covering a larger number of patients treated with higher doses of epirubicin.

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CLINICAL TRIAL

Phase II study of preoperative sequential FEC and docetaxel predicts of pathological response and disease free survival

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Abstract *Purpose* This multicenter phase II study examined the impact of pathological effect on survival after preoperative chemotherapy in Japanese women with early stage breast cancer. *Patients and methods* Prior to surgery, patients received four cycles of FEC (fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2, cyclophosphamide 500 mg/m^2 q3w) followed by four cycles of docetaxel (75 mg/m^2 q3w). Primary endpoint was 3 year disease free survival (DFS) stratified by the absence or presence of Quasi-pCR (QpCR; absence of invasive tumor or only focal residual tumor cells). Secondary endpoints were predictors for QpCR, clinical response, breast conservation rate, and safety. *Results* Between June 2002 and June 2004, 202 women were enrolled. Among 191 assessable patients, 25% achieved QpCR. With 40 months median follow-up, 3 year DFS was estimated at 91% for all patients. 3 year DFS for patients with QpCR was 98% vs. 89% without QpCR (hazard ratio 0.38 [95% Confidence Interval 0.09–0.84], $P = 0.0134$). HER2 status and response to FEC were independent predictors of QpCR. The overall clinical

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response was 75%; 85% of patients achieved breast conservation. Grade 3/4 neutropenia was the most common adverse event, observed in 44% and 35% of patients during FEC and docetaxel, respectively. Treatment related side effects were manageable; there were no treatment related fatalities. *Conclusion* FEC followed by docetaxel is an active and manageable preoperative regimen for women with early stage breast cancer. QpCR following preoperative chemotherapy predicts favorable DFS. HER2 overexpression and clinical response to FEC predict QpCR.

Keywords Clinical trial · Docetaxel · Early stage breast cancer · FEC · Preoperative chemotherapy · Phase II

Introduction

Preoperative systemic chemotherapy has been widely used for patients with operable breast cancer to increase the chance for breast conservation [1–3]. Furthermore, response to preoperative treatment can provide information on long-term survival outcomes. Pathological complete response (pCR) in the breast and axillary lymph nodes predicts a favorable prognosis, whereas non-pCR of the breast or node-positive status does not, which can facilitate tailoring of subsequent treatment [1, 3]. In addition, correlative studies of tumor samples before and after treatment may provide information on markers that could predict response or resistance to treatment [4].

Results from the National Surgical Adjuvant Breast and Bowel Project (NSABP) study B-18 demonstrated the impact of preoperative chemotherapy in patients with operable early stage breast cancer [5]. The protocol-specified anthracycline-containing regimen of four cycles of doxorubicin and cyclophosphamide (AC), resulted in an increased chance of breast-conserving surgery (BCS) compared to no preoperative chemotherapy. The study established pCR as a prognostic marker for long-term disease-free survival and demonstrated that there was no difference in survival whether chemotherapy was administered before or after surgery. Subsequently, studies such as the Aberdeen trial have demonstrated the benefit of the sequential addition of taxanes to preoperative anthracycline regimens [6, 7]. NSABP Protocol B-27 demonstrated that compared to preoperative AC alone, the addition of sequential docetaxel doubled the pCR rate, increased the clinical complete response (cCR) rate, and increased the proportion of patients with negative axillary nodes [3, 7]. Although NSABP B-27 did not show that the addition of docetaxel to AC significantly improved disease free survival (DFS) and overall survival (OS) compared to AC alone, other studies, mainly of patients with node-positive disease, have shown favorable DFS and OS by including a taxane with an anthracycline, either in sequence or combination [8–12]. Multiple neoadjuvant studies demonstrated that patients with pathological complete response to chemotherapy had a good prognosis [1, 2].

Here we conducted a multicenter prospective neoadjuvant trial with four cycles of fluorouracil, epirubicin, and cyclophosphamide (FEC) followed by four cycles of docetaxel in Japanese patients with operable breast cancer to investigate the relationship between pathological effect and survival. The pathological effect was determined using the definitions of Quasi-pCR (QpCR: complete disappearance of invasive carcinoma in the breast or only focal tumor cells remaining in the stroma in the removed breast) [13]. The primary endpoint was to examine 3 year DFS stratified by pathological response (QpCR versus non-QpCR). We also performed a logistic regression analysis to examine which features were associated with QpCR with this regimen. Clinical response, the rate of BCS, and safety were also evaluated.

Methods

Study design and ethics

This multicenter, open-label, single-arm, phase II clinical study was conducted at 13 institutions throughout Japan. This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The protocol was reviewed and approved by the institutional review board of each participating institution and written informed consent was obtained from all patients prior to the study.

Patients

Women aged 20–59 years of age with histologically proven early stage breast cancer (T1c-3 N0 M0/T1-3 N1 M0)

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were enrolled. No prior chemotherapy, radiotherapy, hormonal therapy, or immunotherapy was allowed. Other inclusion criteria were the following: Eastern Cooperative Oncology Group performance status of 0–1; white blood cell count between 4000/mm^3 and 12000/mm^3; neutrophil count ≥2000/mm^3; platelet count ≥100000/mm^3; hemoglobin ≥9.5 g/dl; serum bilirubin <1.25 times upper normal limit (UNL), creatinine <1.5 times UNL, or AST and ALT <1.5 times UNL. Patients with congestive heart failure or left ventricular ejection fraction ≤60% were excluded. Patients were also excluded if they had confirmed infection; serious concomitant illness such as severe cardiovascular disease, uncontrolled diabetes, malignant hypertension and hemorrhagic disease; active concomitant malignancy; brain metastasis; interstitial pneumonia or lung fibrosis confirmed by chest X-ray or computed tomography; pleural or peritoneal effusion that required treatment; pericardial effusion; motor paralysis, peripheral neuropathy or edema history of severe drug allergy; or had previously received long-term corticosteroid therapy. Pregnant or lactating women were also excluded.

Treatment procedures

Four cycles of FEC (fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2, and cyclophosphamide 500 mg/m^2) administered intravenously (i.v.) on day 1 every 21 days were followed by four cycles of docetaxel i.v. (75 mg/m^2) every 21 days, prior to surgery. The doses of docetaxel and epirubicin selected at the time of this study were higher than the approved doses in Japan (60 mg/m^2 each). Premedication consisted of a 5-HT$_3$ antagonist and dexamethasone i.v. on day 1 with oral dexamethasone on days 2 and 3 with each cycle of FEC and dexamethasone i.v. with or without 5-HT$_3$ antagonist on day 1 with each cycle of docetaxel. Administration of recombinant human granulocyte colony-stimulating factor (rh G-CSF) and antibiotics was left to the judgment of each investigator. If patients prematurely discontinued FEC treatment, they were expected to proceed to four cycles of docetaxel.

Treatment could be postponed for a maximum of 2 weeks for severe toxicity. If toxicity did not improve during this period, chemotherapy was discontinued and surgery was recommended. Dose reductions of epirubicin from 100 mg/m^2 to 75 mg/m^2 and for docetaxel from 75 mg/m^2 to 60 mg/m^2 were permitted in case of febrile neutropenia and grade 3 or 4 non-hematological toxicities except for nausea, vomiting, and fatigue. Following chemotherapy and clinical assessment of response, patients underwent surgery. If the tumor was too large or invasive for breast-conserving surgery, modified radical mastectomy was recommended. Sentinel lymph node biopsy (SNB) was performed to confirm disease stage. Most patients with negative biopsies did not undergo surgical clearance of axillary nodes. Autologous or heterologous reconstructive surgery was performed as needed. All patients who underwent breast-conserving surgery were given standard radiotherapy to the remaining ipsilateral breast tissue after surgical recovery. For patients with node-negative status in the sentinel nodes not requiring axillary dissection, radiotherapy to the axilla was allowed but not required. No recommendations were made for post-study hormone therapy in the protocol.

Assessment

Hormone receptor and HER2 overexpression

Estrogen receptor (ER) status and progesterone receptor (PgR) status were determined by immunohistochemistry at each institute. In general, tumors with >10% positively stained tumor cells were classified positive for ER and PgR. HER2 status was also determined at each institute by immunohistochemistry or by fluorescence in situ hybridization (FISH) analysis. HER2 positive tumors were defined as 3+ on immunohistochemistry staining or as positive by FISH.

Central pathological assessment

Haematoxylin and eosin (H&E) and keratin stained slides were prepared as 5 mm tissue sections from the primary tumor. Pathological breast tumor response was assessed by a central review committee consisting of three pathologists using modified criteria of the Japanese Breast Cancer Society [14]. A blinded central review committee evaluated the pathologic response independently to the local pathologists. In this study, the response of stromal invasion and intraductal component was assessed separately. Cytokeratin immunostaining was performed to confirm residual cancer cells in required cases.

Toxicity and clinical assessment

Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria (version 2). Tumor response was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines in patients who had measurable lesions. Tumor and toxicity assessments were performed within 4 weeks prior to FEC treatment, after completion of FEC treatment, and before surgery.

Statistical methods

The primary endpoint was to examine 3 year DFS stratified by pathological response (QpCR versus non-QpCR). Secondary endpoints included predictors for QpCR, clinical response, the rate of BCS, and safety.

For the primary efficacy analysis, we assumed that approximately 25% of patients would achieve QpCR and that the 3 year DFS rate in patients with non-QpCR would be 70%. To demonstrate a 20–25% reduction in the hazard of DFS between patients achieving QpCR compared with those without QpCR, we planned to enroll 200 patients. Using the log rank test this would provide $\alpha = 0.05$ and $\beta = 0.2$.

Kaplan–Meier analysis was used to estimate the values of DFS. DFS was compared using a log-rank test stratified for QpCR and non-QpCR. Events for the calculation of DFS include all local, regional, or distant recurrence, all clinically inoperable and residual disease at surgery, all second cancers, contralateral breast cancers, and all deaths.

In the logistic regression analyses, adjustments were made for the stratification variables of menopausal status, tumor size, estrogen receptor status, progesterone receptor status, HER2 status, clinical response to FEC treatment and clinical response to docetaxel following FEC treatment. Analyses were performed with JMP (version 6, SAS Institute Inc.). Analyses of endpoint data reported here are based on information received as of July 2007.

Results

Patient characteristics

Between June 2002 and June 2004, 202 patients were prospectively enrolled. As two patients were ineligible and two patients withdrew consent, 198 patients were assessed for safety. One patient was removed from the study after planned chemotherapy but before surgery because of a protocol violation (non-protocol chemotherapy), four patients elected to not have surgery and withdrew from the study, and two were lost to follow-up, leaving 191 evaluable for clinical, pathologic assessment and DFS.

The median age of the assessable 198 patients was 46 years, and 72% of patients were pre-menopausal. The majority of the patients had T2 tumors (74%), with 20% of the patients having T3 tumors and 6% with T1 tumors (Table 1). Distribution with regard to hormone receptor or HER2 overexpression was representative of that seen in common practice in Japan [15].

Table 1 Patients characteristics ($n = 198$)

	No. of patients	%
Age (years)		
Median	46	
Range	25–60	
Menopausal status		
Pre	142	72
Post	56	28
Tumor stage		
T1	12	6
T2	146	74
T3	40	20
Nodal stage		
N0	80	40
N1	117	59
N2	1	1
Hormone receptor status		
ER		
Positive	133	67
Negative	62	31
Unknown	3	2
PgR		
Positive	100	51
Negative	95	48
Unknown	3	2
HER2 (IHC)		
0	60	30
1+	54	27
2+	42	21
3+	38	19
Unknown	4	2

ER estrogen receptor, *PgR* progesterone receptor, *IHC* immunohistochemistry

Percentages may not add up to 100% because of rounding

Compliance to chemotherapy and toxicity

Dose reduction due to toxicities was made in 18% of the patients during FEC treatment; febrile neutropenia (19), grade 3–4 neutropenia without fever (10), suspicion of febrile neutropenia (4), vomiting, and deterioration in liver function (1 each) and 14% of patients during docetaxel therapy, febrile neutropenia (5), grade 3–4 neutropenia without fever (5), neutropathy (2), deterioration in liver function (2), myalgia (2) allergy (1) previous reduction of FEC (8), and unknown (2).

Six patients (3%) discontinued FEC treatment due to toxicities (3: two patients with febrile neutropenia and one with vomiting), progression of disease (2), and mental disorder (1). Ten (please refer toxicity section) patients (5%) discontinued docetaxel treatment due to toxicity (3:

one patient each with rash, febrile neutropenia, and phototoxicity), progression of disease (3), and patients' requests for early surgery (2) changing hospital (1), patient's request (1).

Percentage of treatment cycles requiring dose reduction for FEC, docetaxel and all were 11.1, 11.6 and 11.3%. Percentage of treatment cycles (FEC, docetaxel and all) including rh G-CSF were 10.5, 8.2 and 9.4%, respectively.

The safety profile is summarized in Table 2. Four patients didn't receive docetaxel treatment at patients' request. For toxicity 198 and 194 patients were evaluable for FEC treatment and docetaxel treatment, respectively. The most common adverse event was grade 3 or 4 neutropenia, which was observed in 44% of patients during FEC treatment and 35% of patients during docetaxel treatment. Fever, including febrile neutropenia, was seen in 20% and 7% during treatment with FEC and docetaxel, respectively. The only grade 3–4 non-hematologic toxicities reported were; nausea (12 patients), vomiting (11) and fatigue (3). No fatal events were observed.

Response to treatment

The overall clinical response was 74% (95% CI, 67–80%) with 22% CR and 52% PR. Thirty-eight (51%) of 75 FEC non-responders had a response to docetaxel treatment. One hundred and six of 118 FEC responders maintained their response or had a continued decrease in tumor size with docetaxel (Table 3). QpCR were seen in 25% of patients (including 16% complete disappearance of invasive carcinoma in the breast). One patient was removed from assessable for BCS because of a protocol violation. BCS was achieved in 85% of all the assessable patients. Ninety-two percent of patients who had original tumor size 3 cm or less underwent BCS; those with larger tumors had an 80% rate of BCS. As of July 11, 2007, with a median follow up of 40 months, the estimated 3-year DFS was 91% for all patients. Patients who achieved QpCR had significantly improved DFS compared to those without QpCR (QpCR (98%) and non-QpCR (89%), log rank test, $P = 0.0333$, Fig. 1). HR 0.38 [95% CI 0.09–0.84], $P = 0.0134$).

Predictive factors of pathological response

A multiple logistic regression analysis was performed to examine which factors among menopausal status, tumor size, estrogen receptor status, progesterone receptor status, HER2 status and clinical response to FEC were associated with QpCR (Table 4). HER2 status and response to the initial FEC treatment and response to docetaxel were independent predictive factors for QpCR. The QpCR rates stratified by HER2 and ER are shown in Fig. 2. QpCR rate was 67, 33, 35 and 13% in HER2 positive/ER negative, HER2 positive/ER positive, HER2 negative/ER negative, HER2 negative/ER positive, respectively.

Table 2 Treatment related toxicities

	FEC (n = 198)		Docetaxel (n = 194)	
	All grades n (%)	Grade 3, 4 n (%)	All grades n (%)	Grade3, 4 n (%)
Non-hematologic toxicities				
Fatigue	83 (42%)	2 (1%)	83 (42%)	1 (1%)
Diarrhea	17 (9%)	1 (1%)	31 (16%)	0
Nausea	162 (82%)	11 (6%)	81 (42%)	1 (1%)
Vomiting	98 (50%)	10 (5%)	38 (20%)	1 (1%)
Neurotoxicity	6 (3%)	0	85 (44%)	2 (1%)
Constipation	67 (34%)	0	50 (26%)	1 (1%)
Arthralgia/myalgia	12 (6%)	0	60 (30%)	1 (1%)
Hematologic toxicities				
Hemoglobin	119 (60%)	1 (1%)	101 (52%)	0
Platelets	26 (13%)	1 (1%)	3 (2%)	1 (1%)
AST/ALT	81 (41%)	3 (2%)	70 (36%)	1 (1%)
Leukocytes	131 (66%)	68 (35%)	92 (47%)	57 (30%)
Neutrophils	137 (69%)	85 (44%)	85 (44%)	67 (35%)
Febrile neutropenia	–	40 (20%)	–	14 (7%)

FEC fluorouracil, epirubicin, cyclophosphamide

Table 3 Clinical response after FEC and after docetaxel following FEC treatment (n = 194)

Clinical response, N (%)	Overall	
	Responder	Non-responder
FEC		
Responder	106 (90%)	13 (10%)
Non-responder	38 (51%)	37 (49%)

cCR + cPR responder, *cSD + cPD* non-responder, *FEC* fluorouracil, epirubicin, cyclophosphamide, *CI* confidence interval

Discussion

We have presented results from the largest study to date that enrolled Japanese women undergoing preoperative chemotherapy for early stage breast cancer. Our findings demonstrated that four cycles of preoperative FEC followed by four cycles of docetaxel conferred a high rate of BCS, even among patients with primary tumors larger than 3 cm. We found a significant improvement in DFS when QpCR could be achieved, compared to the absence of QpCR. HER2 overexpression, response to FEC and response to docetaxel were significant predictors of QpCR with this regimen.

Regarding toxicity, there were no fatal events and no significant differences in the types and severity of toxicity as compared to other recent studies using similar regimens outside of Japan [6, 8, 9, 16–18]. Compared with overseas studies that also did not allow rh G-CSF the incidence of fever was the same in this study [8, 19]. In another studies which showed lower incidence of febrile neutropenia (13.5%) all patients were treated with rh G-CSF [16].

One of the merits of neoadjuvant chemotherapy for operable breast cancer is to decrease the size of the primary tumor in order to allow for BCS. The study protocol did not provide guidelines for breast conservation; therefore, the BCS rate that we observed reflected the biases that may occur in real-life clinical practice in Japan. Nevertheless, the BCS rate of 80% that we observed was favorable compared with other neoadjuvant studies performed overseas [3, 16].

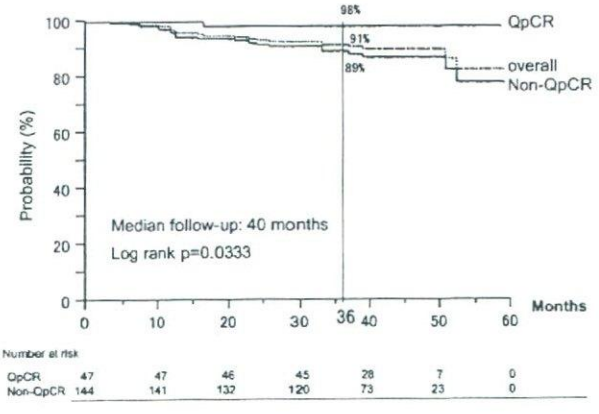

Fig. 1 Relationship of QpCR and non-QpCR to disease free survival

The PACS 01 trial which compared six cycles of adjuvant FEC with a sequential regimen of three cycles of FEC followed by three cycles of docetaxel 100 mg/m^2 (FEC-D) demonstrated an 18% risk reduction in DFS and 27% risk reduction in OS with FEC-D (adjusted P = 0.017). This study supports the conclusions that sequential adjuvant chemotherapy with FEC followed by docetaxel significantly improves DFS and OS in node-positive breast cancer patients [9]. In the current study the dose of docetaxel 75 mg/m^2 was selected based on the recommended doses for docetaxel in Japan, and we showed that the actual 3-year DFS rate of 91% was better than expected based on the results of overseas studies [7, 9, 20]. This confirms that the approved doses of 75 mg/m^2 is an appropriate does in Japanese women.

Furthermore a new definition of QpCR was defined for pathological effect in this study. When stratified between QpCR and non-QpCR, patients with QpCR had significantly favorable DFS. Indeed by adding docetaxel to FEC patients with QpCR resulted in improved survival similar to previous studies.

Even without anti-HER2 targeting therapy, a QpCR rate >60% was achievable in ER negative and HER2 positive tumors. A multivariate analysis has indicated the significant value of HER2 overexpression, which seems to suggest the importance of HER2 in the prediction of QpCR with this regimen. In this study both an anthracycline and docetaxel were used, so it is not clear which treatment was more strongly associated with HER2 as a predictive value of QpCR. Data in the metastatic and adjuvants setting suggest that docetaxel regimens may be more active than non docetaxel regimens in HER2 positive tumors [8, 21]. The value of HER2 status as a predictor of response to anthracycline-based chemotherapy is still a matter debate. On the other hand, there are several implicative data showing the predictive value of topoisomerase (Topo)-II for anthracyclines because Topo-II is a molecular target of anthracyclines [22–25]. There is evidence that HER2 amplification and Topo-II amplification usually occur in parallel and it is rare to have Topo-II amplification without HER2 amplification [23, 26]. In this study QpCR rate might clarify the difference between HER2 positive tumors and HER2 negative tumors. No patient has received trastuzumab in the adjuvant setting. Future translational studies will be necessary to explore the significance of Topo-II amplifications as well as HER2 gene amplifications in the prediction of the pathological response of this regimen. This result will be included the information in the future if

Table 4 Predictive variables for QpCR

Variables	Before treatment OR 95% CI (*P*)	After FEC treatment OR 95% CI (*P*)	After docetaxel following FEC treatment OR 95% CI (*P*)
Menopausal status	1.43	1.38	1.37
Pre (versus post)	0.94–2.15 (NS)	0.89–2.14 (NS)	0.87–2.12 (NS)
Tumor size	0.89	0.93	0.87
>3 cm (vs ≤3 cm)	0.61–1.3 (NS)	0.63–1.37 (NS)	0.59–1.28 (NS)
ER	1.4	1.44	1.35
Negative (versus Positive)	0.87–2.27 (NS)	0.88–2.36 (NS)	0.81–2.23 (NS)
PgR	1.61	1.49	1.65
Negative (versus Positive)	0.97–2.67 (NS)	0.89–2.51 (NS)	0.98–2.79 (NS)
HER2	2.02	2.24	2.11
3+ (vs <3+)	1.31–3.11 (0.0014)	1.42–3.53 (0.0005)	1.36–3.3 (0.0009)
Clinical response to FEC treatment	–	1.78	–
Response (versus non-response)	–	1.15–2.76 (0.0096)	–
Clinical response to docetaxel following FEC treatment	–	–	1.99
Response (versus non-response)	–	–	1.14–3.47 (0.0154)

QpCR quasi pathological complete response, *FEC* fluorouracil, epirubicin, cyclophosphamide, *OR* odds ratio, *ER* estrogen receptor, *PgR* progesterone receptor, *CI* confidence interval, *NS* not significant

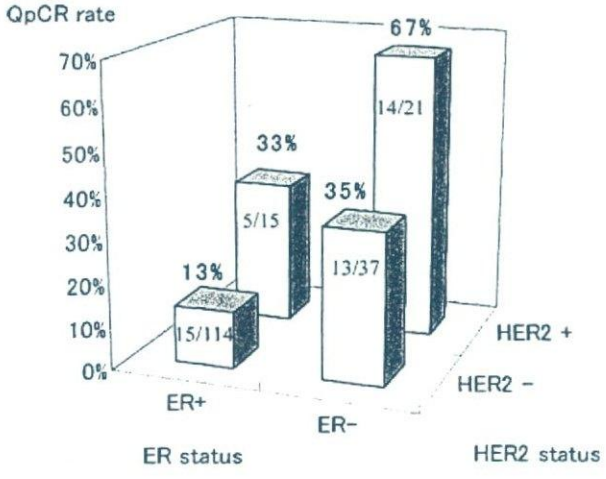

Fig. 2 Relationship between QpCR and HER2/ER status (n=187)

we use anthracycline and trastuzumab for all HER2 positive patients.

In the present study, though a multivariate analysis hasn't indicated the significant value of the status of hormone receptor, QpCR rate was higher in ER negative tumors than ER positive tumors, and QpCR rate in ER negative and HER2 positive tumors was remarkably high compared with ER positive and HER2 negative tumors. This model suggests that ER status is a dependent predictor, for QpCR possibly because it is related to HER2 expression. The sample size was perhaps too small to effectively determine the true impact of ER negative status as a predictor of QpCR. As most patients who are HER2 positive are also ER negative, it is likely that ER status will have some predictive value. However, larger studies are needed to determine this. These results are important for considering individual preoperative systemic therapy. This trend was similar to previous studies using AC followed by paclitaxel regimens, though the therapeutic situations are different [10, 12, 27, 28]. According to recent meta-analyses of post-operative adjuvant therapy, chemotherapy including cyclophosphamide/methotrexate/5FU (CMF)-type regimens, anthracycline-containing regimens and anthracycline followed by paclitaxel are more effective for hormone receptor negative tumors than for hormone receptor positive tumors [10–12, 27–32]. However, while hormone receptor negative tumors may be more responsive to preoperative regimens, a survival benefit can be observed regardless of receptor status [2]. In this study a multivariate analysis hasn't indicated the significant value of the status of hormone receptor. This may be affected by addition of docetaxel. Dose response with anthracycline is also different between hormone receptor positive tumors and hormone receptor negative tumors. For ER negative tumors, higher anthracycline doses may be required for improved prognosis, however, for ER positive tumors it might not be necessary [29].

In this study, most tumors responded to docetaxel even if they did not respond to FEC. However, some tumors showed a response to the initial therapy but a lesser response to the second therapy. This underscores the need to include non-cross resistant treatments in the

management of early stage breast cancer [33]. Various non-cross resistance molecules may be involved in this clinical phenomenon. Recent investigations indicate that initial chemotherapy may change the phenotype of the tumor by inducing pro-survival molecules in tumor cells or stroma [2, 3, 5, 7, 16]. In particular, key mediators such as nuclear factor-kappa B, cyclooxygenase-2 and thymidine phosphorylase are known to be induced by chemotherapy frequently, which may change those tumors relatively anti-apoptotic to the second chemotherapy [34–36]. From the clinical point of view, it would be useful to modify the treatment schedule based on initial response to treatment. Since the types of pro-tumor molecules and the magnitude of induction are different between agents, it might be reasonable to consider a different sequence (taxane followed by anthracycline), if information on the tumor phenotype could be obtained before starting treatment. Various treatment scenarios for non-responders to FEC could be considered. According to recent study results, surgery might be an option for non-responders to initial anthracyclines [37]. In order to enhance the effect of docetaxel, the combination with fluoropyrimidines such as capecitabine may be an option. Obviously for HER2 overexpressing tumors, anti-HER2 containing therapy should be considered. For the ER positive and HER2 negative phenotype, hormone therapy might be an option if tumors are relatively well differentiated. Individual treatment based on ER/HER2 status and the clinical response to the initial anthracyclines may be integrated as future direction [37].

In conclusion, 8-cycle preoperative chemotherapy with non-cross resistant regimens, FEC followed by docetaxel, is safe, feasible, and effective as primary systemic therapy for women with early stage breast cancer. In particular, the regimen allows a majority of Japanese patients to avoid the need for mastectomy. Patients with QpCR demonstrated significantly superior survival results. HER2 over-expression, response to FEC and response to docetaxel were significant predictors for QpCR. Based on our results, preoperative FEC followed by docetaxel should be considered a standard option for the treatment of Japanese women with operable breast cancer.

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原著

2008.2.1受付

原発乳癌に対するFEC followed by docetaxel 100mg/m² 併用療法による術前化学療法の検討—JBCRG02—

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JBCRG conducted a multicenter prospective neoadjuvant trial with 4 cycles of FEC followed by 4 cycles of docetaxel 100 mg/m² in women with primary operable breast cancer to evaluate efficacy and safety. Between August 2004 and July 2006, 50 patients were enrolled. Pathological complete response (pCR) as primary endpoint was 27%. Quasi-pCR was 31% giving an overall clinical response of 67%, with breast conservation surgery rate of 82%. 84% of patients completed 8 cycles of chemotherapy and 52% of patients completed all cycles without dose reduction. The common adverse events during docetaxel treatment were grade 3 diarrhea, neurotoxicity, edema, myalgia and arthralgia, which responded to appropriate supportive care. Preoperative FEC followed by docetaxel 100mg/m² for primary operable breast cancer was effective and tolerated with appropriate supportive care and reduction for Japanese patients.

Key words : Primary systemic chemotherapy, Clinical trial, FEC, Docetaxel
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はじめに

乳癌治療においては，手術とならび放射線治療や，ホルモン療法，epirubicinなどアンスラサイクリン系薬剤とdocetaxelなどタキサン系薬剤などの化学療法，さらに分子標的薬剤などを組み合わせた薬物治療が行われている．転移・再発乳癌に対する治療の目的は，生存期間の延長，症状緩和によるQOLの改善であり，患者の状態をみながら治療方針を決定していくことが重要である．一方，原発乳癌に対する薬物療法の目的は，全身治療による再発予防であり，根治を目指し早期に全身治療を行うことで再発や死亡リスクを軽減させることが海外の多くの臨床試験から証明されている[1]．

Wolmarkらは，doxorubicin/cyclophos-

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phamide (AC) を用い，術前化学療法と術後化学療法の比較したNSABP B-18において，生存に関して両群に差は認められず，術前化学療法によりpathological complete response (pCR) が得られた症例のみ生存期間，無病生存期間ともに明らかな予後の改善が認められたことを報告した[2]．この試験以降，術前化学療法では，薬剤の感受性を直接知ることができ，ある程度の予後予測ができること，down stagingにより乳房温存の適応が拡大することなどが明らかになり，多くの術前化学療法試験が行われてきている．

また，NSABP B-27では，Bearらは，術前化学療法においてAC 4コースにdocetaxel 100mg/m²を4コース逐次投与追加した群 (AC-docetaxel) がAC単独群と比較し，有意にpCR率が高い結果が得られたことを報告した[3]．

われわれJapan Breast Cancer Research Group (JBCRG) ではアンスラサイクリン系薬剤としてdoxorubicinよりも心毒性が少ないepirubicinを，タキサン系薬剤としてdocetaxel 75mg/m²を選択し，原発乳癌に対するfluorouracil 500mg/m²/epirubicin 100mg/m²/cyclophosphamide 500mg/m² (FEC) followed by docetaxel 75mg/m²併用療法による術前化学療法 (FEC-DOC75) を検討するJBCRG01試験を行い，pCRや臨床効果などの有効性と安全性を確認した[4]．そこで次の段階として，docetaxelの用量を海外における原発乳乳癌に対して使用されている100mg/m²にすることにより，原発乳癌に対しより高いpCRが得られることを期待し，primary endpointを病理組織学効果として原発乳癌に対するFEC followed by docetaxel 100mg/m²併用療法による術前化学療法 (FEC-DOC100) を検討するJBCRG02試験を行った．

1. 対象と方法

1) 試験の目的

切除可能な原発乳癌症例 (T1c-3N0M0/T1-3N1M0) を対象に，FEC 4コース投与後docetaxel 100mg/m²を4コース投与する併用療法による術前化学療法を行い，有効性と安全性を検討した．

Primary endpointは病理組織学的効果，secondary endpointは安全性 (有害事象，治療完遂率)，抗腫瘍効果，乳房温存術施行率とした．

2) 対象症例

切除可能な原発性乳癌症例 (T1c-3N0M0/T1-3N1M0) のうち，以下の基準を満たす症例を対象とした．①組織学的に浸潤性乳癌と診断された症例，②前治療として化学療法，放射線療法，内分泌療法，免疫療法が行われていない症例，③Performance Status (P.S.) が0～1の症例，④測定可能病変を有する症例，⑤主要臓器の機能が十分に保持されており，以下の基準を満たす症例．ヘモグロビン9.5g/dL以上，白血球数4,000/mm³以上，12,000mm³以下，好中球数2,000/mm³以上，血小板数100,000/mm³以上，血清総ビリルビン値施設基準値上限の1.25倍以下，AST，ALT施設基準値上限の1.5倍以下，血清クレアチニン値施設基準値上限の1.5倍以下，ejection fraction 60%以上，心電図正常．⑥年齢20歳以上60歳未満の症例，⑦本試験の参加について被験者本人の同意が文書にて得られた症例．

なお以下の基準に該当した場合は除外した．①薬剤過敏症の既往歴のある症例，②重篤な合併症を有する症例（例えば，悪性高血圧，うっ血性心不全，冠不全，6カ月以内の心筋梗塞，治療を要する不整脈，感染症，出血傾向の発現等），③発熱を有し，感染の疑われる症例，④末梢神経症状を有する症例，⑤治療を要する胸水・心嚢水貯留例，⑥活動性の重複癌を有する症例，⑦炎症性乳癌症例，⑧両側乳癌の症例，⑨男子乳癌の症例，⑩妊婦，授乳婦および妊娠の可能性（意志）のある女性，⑪浮腫のある症例，⑫水痘症の症例，⑬胸部単純X線写真およびCTで明らかな間質性肺炎，または肺線維症のある症例，⑭あらかじめステロイドの治療を必要とする症例，⑮精神疾患既往，または治療中の症例，⑯その他，試験責任医師が不適当と判断した症例．

3) 投与方法

選択基準を満たす切除可能な原発乳癌患者に対してFEC (fluorouracil 500mg/m², epirubicin 100mg/m², cyclophosphamide 500mg/m²) を3週間