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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. The numbers of patients in each subgroup stratified by Cav-1 grade.

Table S2. Univariate analyses of Cav-1 expression associated with disease-free survival of the training and testing cohort.

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Docetaxel Followed by Fluorouracil/Epirubicin/Cyclophosphamide as Neoadjuvant Chemotherapy for Patients with Primary Breast Cancer

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Objective: This multicenter, open-label, single-arm, Phase II study assessed the efficacy of a neoadjuvant chemotherapy with docetaxel (75 mg/m^2 q3w) followed by 5-fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2 and cyclophosphamide 500 mg/m^2 q3w in patients with early-stage breast cancer.

Methods: Women with resectable breast cancer (T1c–3 N0 M0 or T1–3 N1 M0) were enrolled. Before surgery, patients received four cycles of docetaxel followed by four cycles of 5-fluorouracil, epirubicin, and cyclophosphamide. The primary endpoint was the pathological complete response (pCR) rate defined for the breast alone, assessed by a central review committee. Secondary endpoints included clinical response and safety.

Results: One hundred and thirty-seven patients were enrolled. Of the 132 patients assessable for pathologic response, 23% (95% confidence interval, 16–31%) experienced a pathological complete response and 6% (95% confidence interval, 3–12%) had a near pathological complete response (few remaining cancer cells), resulting in a quasi-pathological complete response of 29% (95% confidence interval, 21–37%). Clinical response rate following the initial docetaxel regimen was 64%. The overall clinical response rate after completion of 5-fluorouracil, epirubicin, and cyclophosphamide was 79%; breast-conserving surgery was performed in 79% of patients. More patients with triple-negative disease (estrogen/progesterone receptors negative; human epidermal growth factor 2 negative) experienced a pathological complete response [14/29, (48%); 95% confidence interval, 29–68%] versus those with other molecular subtypes. The safety profile was acceptable.

Conclusions: Eight cycles of neoadjuvant chemotherapy—docetaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide—are tolerable and conferred high rates of pathological complete response and breast-conserving surgery. Patients with triple-negative disease were more likely to achieve pathological complete response versus other subtypes, suggesting that selecting appropriate neoadjuvant chemotherapy based on molecular subtype could be possible.

Key words: breast neoplasms – neoadjuvant therapy – FEC protocol – docetaxel

INTRODUCTION

Neoadjuvant chemotherapy has been widely used for patients with operable breast cancer to increase the chance of breast conservation (1–7). Furthermore, response to neoadjuvant treatment can provide important information on long-term survival outcomes. Pathological complete response (pCR) in the breast and axillary lymph nodes predicts a favorable prognosis, whereas a lack of pCR in the breast and node-positive status do not (6,7). This implies the possibility of tailoring subsequent treatment according to the response to initial treatment (7–12). In addition, correlative studies of tumor samples before and after treatment may provide information on markers that could predict response or resistance to treatment (13–16).

Results from the National Surgical Adjuvant Breast and Bowel Project (NSABP) Protocol B-18 trial demonstrated the impact of neoadjuvant chemotherapy in patients with operable early-stage breast cancer (17). The protocol-specified anthracycline-containing regimen—four cycles of doxorubicin and cyclophosphamide (AC)—resulted in an increased likelihood of breast-conserving surgery (BCS) compared with no neoadjuvant chemotherapy. The study established pCR as a prognostic marker for long-term disease-free survival (DFS) and demonstrated that there was no difference in survival if chemotherapy was administered before or after surgery. Subsequent studies, such as the Aberdeen trial, have demonstrated the benefit of the sequential addition of taxanes to neoadjuvant anthracycline regimens (5). The NSABP Protocol B-27 trial demonstrated that, compared with neoadjuvant AC alone, the addition of sequential docetaxel doubled the pCR rate, increased the clinical complete response rate (RR) and increased the proportion of patients with negative axillary nodes (7–18).

We previously conducted a Phase II study to evaluate the clinical and pathological response and safety of the FEC regimen (5-fluorouracil, epirubicin and cyclophosphamide) followed by docetaxel as neoadjuvant chemotherapy in Japanese women with early-stage breast cancer [Japan Breast Cancer Research Group (JBCRG) 01 trial]. The results of this study have been reported previously (19). Although the pCR rate was 16% and BCS was possible for 85% of patients, there were some safety concerns, with 18% of patients experiencing febrile neutropenia and 41% of patients experiencing Grade 1/2 peripheral edema (no Grade 3/4 events observed) following the docetaxel regimen (unpublished data). Disease progression occurred in 6% of patients after the completion of all planned treatment (unpublished data).

In an effort to achieve a higher pathological RR with an improved safety profile, we decided to evaluate the efficacy and safety of docetaxel followed by FEC (JBCRG 03 trial)—the reverse of the sequence of chemotherapy used in the JBCRG 01 trial (19). The clinical and pathological effects and the toxicity profile of this regimen are presented here, and the results of predictive marker analyses are discussed.

PATIENTS AND METHODS

Patient Eligibility

This was a multicenter, open-label, single-arm, Phase II study that recruited patients via central registration. Japanese women aged 20–59 years with histologically proven early-stage breast cancer (T1c–3 N0 M0 or T1–3 N1 M0) were enrolled. No prior chemotherapy, radiotherapy, hormonal therapy or immunotherapy was allowed. Other inclusion criteria were Eastern Cooperative Oncology Group performance status 0–1; white blood cell count 4000–12 000/mm^3; neutrophil count $\geq$ 2000/mm^3; platelet count $\geq$ 100 000/mm^3; hemoglobin $\geq$ 9.5 g/dl; serum bilirubin $\leq$ 1.25 times upper limit of normal (ULN); creatinine $\leq$ 1.5 times ULN and aspartate aminotransferase and alanine aminotransferase $\leq$ 1.5 times ULN. Patients with congestive heart failure or left ventricular ejection fraction $\leq$60% were excluded. Patients were also excluded if they had confirmed infection; serious concomitant illness such as severe cardiovascular disease, uncontrolled diabetes, malignant hypertension or hemorrhagic disease; active concomitant malignancy; brain metastasis; peripheral neuropathy; history of edema with severe drug allergy; or previous long-term corticosteroid therapy. Pregnant or lactating women were excluded. Mammography, ultrasonography, magnetic resonance imaging or computed tomography was used to assess the presence of tumors. Baseline evaluations included complete blood cell and platelet count, routine blood chemistry and liver function tests, chest X-ray, bone scan, electrocardiogram and echocardiogram.

The local ethics committee or institutional review board approved the study at each institution. All patients gave written informed consent to participate. The protocol was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

Treatment

Four cycles of docetaxel (75 mg/m^2) administered intravenously (i.v.) every 21 days were followed by four cycles of FEC (5-fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2 and cyclophosphamide 500 mg/m^2) administered i.v. on Day 1 every 21 days before surgery. Premedication was administered based upon each physician's decision to prevent edema, nausea and allergic reactions (e.g. dexamethasone 12 mg i.v. and/or granisetron 4 mg i.v. on Day 1, and oral dexamethasone 8 mg on Days 2 and 3 of docetaxel treatment; dexamethasone 24 mg i.v. on Day 1 and oral dexamethasone 8 mg on Days 2–6 with the FEC regimen). Administration of granulocyte colony-stimulating factor and antibiotics was left to the judgment of each investigator.

Clinical Response Assessment

Tumor assessments were performed within 4 weeks before docetaxel treatment, after completion of docetaxel treatment

and before surgery. Tumor response was assessed using the modified Response Evaluation Criteria in Solid Tumors guidelines (in which confirmatory scans/assessments were not required due to the timing of surgery), for patients who had measurable lesions.

Central Pathologic Assessment

Hematoxylin and eosin-stained slides were prepared from core needle biopsy and surgical specimens from the primary tumor. All surgical specimens were cut in 5 mm interval and all surfaces were microscopically examined in each institution. Pathological response of chemotherapy was assessed by a central review committee consisting of three pathologists who used criteria established by the Japanese Breast Cancer Society. pCR was defined as necrosis and/or disappearance of all tumor cells, and/or the replacement of cancer cells by granulation and/or fibrosis. If only ductal components remained, the pathological response was described as a pCR. Near pCR was defined as extremely high grade marked changes approaching a complete response, with only a few remaining isolated cancer cells (19). Quasi-pCR (QpCR) was the total of both pCR and near pCR. The central review committee evaluated the pathological responses independently from local pathologists. This committee was blinded to the local pathologists' reports. Patients who did not have surgery because of disease progression were considered not to have a pCR.

Hormone Receptor and Human Epidermal Growth Factor 2 Overexpression

Estrogen receptor (ER) and progesterone receptor (PgR) status was determined by immunohistochemistry (IHC) before docetaxel treatment at each participating institute.In general, tumors with more than 10% positively stained tumor cells were classified as positive for ER and PgR. The human epidermal growth factor 2 (HER2) status of the tumor was also determined at each institute by IHC or by fluorescence *in situ* hybridization (FISH) analysis. HER2-positive tumors were defined as those scoring 3+ with IHC staining or testing positive by FISH. HER2-negative tumors were defined as those scoring 0−1+ with IHC or scoring 2+ with IHC and testing negative by FISH.

Surgery and Radiotherapy

Following chemotherapy and clinical assessment of response, patients underwent surgery. If the tumor was too large or invasive for BCS, a modified radical mastectomy was recommended. Careful pathological assessment of tumor margins was performed in accordance with the Japanese Breast Cancer Society criteria (20). Sentinel lymph node biopsy was performed to confirm disease stage or to avoid surgical axillary dissection. Autologous or heterologous reconstructive surgery was performed depending on the patient's requirements and health status. All patients who underwent BCS were given standard radiotherapy to the remaining ipsilateral breast tissue after surgical recovery. For patients diagnosed as sentinel node negative and thus not requiring axillary dissection; radiotherapy to the axilla was allowed.

Toxicity and Dose Modification

Toxicities were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3) throughout treatment with docetaxel and FEC before surgery. Treatment could be postponed for a maximum of 2 weeks only for severe toxicity. If the adverse event (AE) did not improve during this period, chemotherapy was discontinued and surgery was recommended. Dose reductions were permitted for docetaxel from 75 to 60 mg/m^2 and for epirubicin from 100 to 75 mg/m^2 in cases of febrile neutropenia or Grade 3/4 non-hematologic toxicities, except for nausea, vomiting and fatigue.

Statistical Methods

The primary endpoint was the pCR rate. Before the initiation of the current study, the pCR rate for non-taxane anthracycline regimens ranged from 12.8% (NSABP Protocol B-27) (18) to 15.4% (Aberdeen trial) (5). Previously, we had conducted JBCRG01 trial to evaluate the pCR rate defined for breast disease (19). Therefore, in order to detect improvement in the pCR rate in the same definition of our previous study, a sample of 119 patients was required according to binominal distribution, with a one-sided threshold pCR rate of 12%, an expected pCR rate of 22%, an α error of 5% and a β error of 10%. The target number of patients for recruitment was therefore 119, so assuming that 5% of patients would not be evaluable, we planned to enroll 130 patients. Secondary endpoints included safety, clinical RR, rate of BCS, DFS, overall survival and a subset analysis according to biomarkers. Pathological and clinical RRs were calculated with 95% confidence intervals (95% CIs), with each complete RR based on a binominal distribution. Pathological response was evaluated by hormone receptor status and HER2 status. A multiple logistic regression analysis was performed to examine which factors (menopausal status, tumor size, ER and PgR status, HER2 status and clinical response to docetaxel and FEC) were associated with pCR and QpCR.

RESULTS

Patients Characteristics and Treatment

Enrollment took place from October 2005 through October 2006. One hundred and thirty-seven patients were enrolled. Two patients did not receive study treatment because of early withdrawal of consent; therefore, 135 patients were evaluable for safety and clinical response. These evaluable

Table 1. Patients' characteristics

Characteristic	Value[a]
Number of evaluable[b] patients	135
Age (years)	
Median	46
Range	24–62
Performance status, *n* (%)	
0	133 (99)
1	2 (1)
Menopausal status, *n* (%)	
Premenopausal	94 (70)
Postmenopausal	41 (30)
Clinical tumor stage, *n* (%)	
T1	13 (10)
T2	98 (73)
T3	24 (18)
Clinical nodal stage, *n* (%)	
N0	62 (46)
N1	73 (54)
ER status, *n* (%)	
Positive	86 (64)
Negative	46 (34)
Unknown	3 (2)
PgR status, *n* (%)	
Positive	63 (47)
Negative	70 (52)
Unknown	2 (1)
HER2 status,[c] *n* (%)	
0	21 (16)
1+	63 (47)
2+	20 (15)
3+	31 (23)

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PgR, progesterone receptor.
[a]Percentages may not add up to 100% because of rounding.
[b]Number of patients evaluable for safety and clinical response.
[c]Evaluated by immunohistochemistry.

patients included two patients aged 60 and 62 years (included because their age was not considered to influence the evaluation). Two patients were lost to follow-up before surgery, thus 133 patients were evaluable for surgical response. A total of 132 patients were evaluable for pathological response; one patient was excluded owing to lack of confirmation of invasive carcinoma (following the pathologic central review) due to inadequate samples from core needle biopsy before study treatment.

The patient characteristics are summarized in Table 1. Thirty patients (22%) had triple-negative disease, defined as ER-negative, PgR-negative and HER2-negative primary breast cancer, including one patient who was lost to follow-up before surgery.

Overall, 98 patients (73%) completed the planned eight cycles of treatment without dose reductions or study discontinuation. A total of 115 (85%) and 106 (82%) patients completed all four planned treatment cycles of docetaxel and FEC, respectively; dose reductions were necessary in 9 (7%) and 17 (13%) patients, respectively. The majority of the dose reductions were attributable to toxicities, particularly febrile neutropenia during treatment with FEC (10 versus 2 patients during docetaxel treatment). Dose reductions due to neutropenia were required by three patients each during the docetaxel and FEC regimens. Eleven (8%) and six patients (5%), respectively, discontinued treatment during docetaxel and FEC therapy because of toxicities (five patients discontinued during both regimens) or disease progression (six patients during docetaxel and one patient during FEC). The mean dose intensities were 24.2 and 30.3 mg/m^2/week for docetaxel and epirubicin, respectively.

Toxicities

The incidence of treatment-related AEs is summarized in Table 2. Neutropenia was the most common Grade 3/4 treatment-related AE and was observed in 44% and 60% of patients during docetaxel and FEC therapy, respectively. Overall, 67% and 15% of patients experienced at least one episode of Grade 3/4 neutropenia or febrile neutropenia, respectively. For non-hematologic toxicities of any grade, rash, sensory neuropathy, edema, muscle pain and joint pain occurred more frequently during docetaxel treatment than with FEC. Conversely, the frequency of gastrointestinal symptoms, such as nausea, vomiting and anorexia, was higher with FEC than with docetaxel. The frequency of Grade 1/2 peripheral edema was similar during exposure to docetaxel (33%) and FEC (29%); no patient had Grade 3/4 edema. Grade 3/4 non-hematologic toxicities, including gastrointestinal disturbances, were infrequent during both docetaxel and FEC. No fatal AEs were reported.

Clinical Response to Treatment

The overall clinical RR was 79% (106/135; 95% CI, 71–85%), with a clinical complete RR of 21% (29/135), a partial RR of 57% (77/135) and a disease progression rate of 5% (7/135). The clinical RR following the initial docetaxel regimen was 64%. The clinical responses to treatment with docetaxel followed by FEC according to response to initial docetaxel are shown in Table 3. Eight of the 135 patients (6%) progressed during docetaxel administration; 2 of 135 patients (1%) had disease progression during FEC. Of the 30 patients with triple-negative disease, 7 patients were observed to have disease progression following docetaxel treatment. One of the 17 patients with ER-positive, PgR-negative and HER2-negative tumors had disease

Table 2. Treatment-related adverse events

Adverse event, *n* (%)	DOC ($n = 135$)		FEC ($n = 29$)		Overall ($n = 35$)	
	All grades	Grade 3/4	All grades	Grade 3/4	All grades	Grade 3/4
Non-hematologic toxicities						
Infection with neutropenia	6 (4)	2 (1)	3 (2)	2 (2)	9 (7)	4 (3)
Fever	15 (11)	0	13 (10)	1 (1)	22 (16)	1 (1)
Infection (other)	3 (2)	1 (1)	2 (2)	0	4 (3)	1 (1)
Fatigue	82 (61)	0	84 (65)	2 (2)	98 (73)	2 (1)
Nausea	52 (39)	1 (1)	102 (79)	3 (2)	108 (80)	4 (3)
Vomiting	19 (14)	1 (1)	51 (40)	3 (2)	61 (45)	4 (3)
Anorexia	53 (39)	1 (1)	86 (67)	2 (2)	91 (67)	2 (1)
Stomatitis	50 (37)	1 (1)	51 (40)	0	68 (50)	1 (1)
Diarrhea	39 (29)	1 (1)	20 (16)	0	46 (34)	1 (1)
Phlebitis	2 (1)	1 (1)	2 (2)	0	4 (3)	1 (1)
Alanine aminotransferase	36 (27)	0	50 (39)	2 (2)	57 (42)	2 (1)
Aspartate aminotransferase	19 (14)	0	34 (26)	1 (1)	40 (30)	1 (1)
Nail changes	2 (1)	0	33 (26)	1 (1)	33 (24)	1 (1)
Weight loss	5 (4)	0	6 (5)	1 (1)	8 (6)	1 (1)
Creatinine	4 (3)	1 (1)	6 (5)	0	7 (5)	1 (1)
Edema	44 (33)	0	37 (29)	0	55 (41)	0
Hematologic toxicities						
Neutropenia	60 (44)	59 (44)	91 (71)	77 (60)	100 (74)	91 (67)
Leukopenia	69 (51)	50 (37)	101 (78)	66 (51)	108 (80)	76 (56)
Thrombocytopenia	13 (10)	0	28 (22)	2 (2)	31 (23)	1 (1)
Anemia	66 (49)	0	99 (77)	1 (1)	106 (79)	1 (1)
Febrile neutropenia	9 (7)	9 (7)	15 (12)	15 (12)	20 (15)	20 (15)

DOC, docetaxel; FEC, 5-fluorouracil, epirubicin and cyclophosphamide.

Table 3. Clinical response to DOC followed by FEC according to response to initial DOC treatment ($n = 135$)

Clinical response,[a] *n* (%)	Total[b]	Responder	Non-responder
Response to DOC			
Responder	87 (64)	79 (58)	8 (6)
Non-responder	48 (36)	27 (20)	21 (16)

[a]Overall response was confirmed after completion of chemotherapy in comparison with before docetaxel treatment.
[b]Percent value of each column was calculated by dividing by the total number of the evaluable patients ($n = 135$).

progression; while of the 53 patients with ER-positive, PgR-positive, and HER2-negative tumors and of the 9 patients with ER-positive, PgR-positive, and HER2-positive tumors, no patient had disease progression during docetaxel treatment. Among those with triple-negative disease, the majority of patients with disease progression after initial docetaxel were premenopausal [6/7 patients (86%)] and had solid-tubular carcinoma which characterized by solid cluster of cancer cells with expansive growth forming sharp borders [4/7 patients (57%)], as assessed using the Japanese Breast Cancer Society histological classification of breast tumors (21) (Table 4). Excluding the differences outlined above, there were no differences between patient and tumor characteristics for those with progressive disease versus non-progressive disease.

Twenty-seven of 48 non-responders to docetaxel (56%) had a response to FEC treatment; however, 8 of 87 responders to docetaxel (9%) showed no improvement in response with FEC treatment. Following chemotherapy, BCS was performed for 105 of 133 assessable patients (79%).

Pathological Response and Predictive Factors to Treatment

The primary endpoint—pCR rate—was 23% (95% CI, 16–31%). A near pCR rate of 6% (95% CI, 3–12%) resulted

Table 4. Clinical and pathologic characteristics of triple-negative breast cancer[a] for patients with progressive disease versus patients without progressive disease, following initial docetaxel therapy

Characteristic	Without PD	PD
No. of evaluable patients	23	7
Age, years		
Median	43	46
Range	(30–62)	(29–53)
Menopausal status, *n* (%)		
Premenopausal	15 (65)	6 (86)
Postmenopausal	8 (35)	1 (14)
Tumor stage		
T1	2 (9)	0
T2	14 (61)	5 (71)
T3	7 (30)	2 (29)
Nodal stage, *n* (%)		
N0	13 (57)	3 (43)
N1	10 (43)	4 (57)
Tumor type, *n* (%)		
Solid-tubular carcinoma	6 (26)	4 (57)
Papillotubular carcinoma	5 (22)	3 (43)
Scirrhous carcinoma	3 (13)	0
Unspecified invasive carcinoma	9 (39)	0

PD, progressive disease.
[a]Triple-negative tumors were defined as ER-negative, PgR-negative and HER2-negative primary breast cancer.

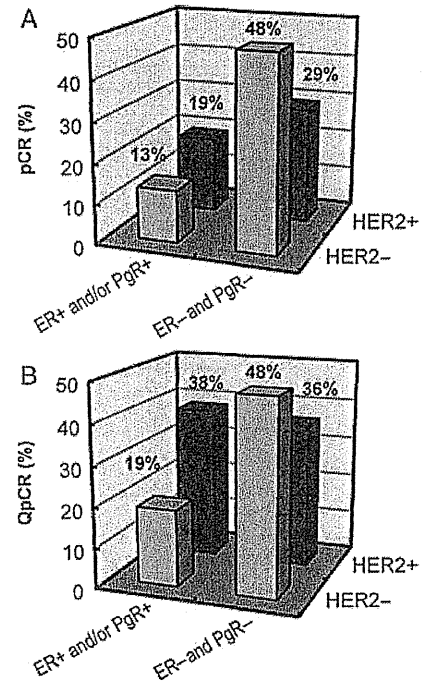

Figure 1. (A) Relationship between pCR versus HER2 and ER/PgR status following DOC and FEC ($n = 129$). (B) Relationship between QpCR versus HER2 and ER/PgR status following DOC and FEC ($n = 129$). Three patients were excluded from evaluable patients for pathologic response ($n = 132$) because of their unknown hormone receptor status. There were no near pCR case observed in triple-negative (ER−, PgR− and HER2−) diseases. DOC, docetaxel; ER, estrogen receptor; FEC, 5-fluorouracil, epirubicin, and cyclophosphamide; HER2, human epidermal growth factor receptor 2; pCR, pathologic complete response; PgR, progesterone receptor; QpCR, quasi-pathologic complete response.

in a QpCR rate of 29% (95% CI, 21–37%) when combined with the pCR. Pathological response of each subset population according to their hormone receptor and HER2 status is summarized in Fig. 1A and B. Patients with triple-negative disease had the highest pCR rate of 48% (95% CI, 29–68%). Near pCR was not observed in triple-negative disease. Patients with HER2-positive, ER-negative and PgR-negative tumors had a pCR rate of 29% (95% CI, 8–58%) and a QpCR rate of 36% (95% CI, 13–65%); patients with HER2-positive and ER-positive and/or PgR-positive tumors had a pCR rate of 19% (95% CI, 4–46%) and a QpCR rate of 38% (95% CI, 15–65%). Patients with HER2-negative and ER-positive and/or PgR-positive tumors had the lowest pCR and QpCR rates (13%; 95% CI, 6–23% and 19%; 95% CI, 10–30%, respectively). One of the seven patients who experienced clinical disease progression with initial docetaxel treatment had a QpCR following FEC.

The relationship between tumor pathological feature and pCR rate is shown in Table 5. The only variable found to be significantly associated with a pCR after docetaxel treatment was ER status.

Survival outcomes will be reported when the 5-year follow-up has been completed for this study.

DISCUSSION

This is the first report to evaluate the effectiveness of an initial docetaxel regimen for neoadjuvant therapy of Japanese patients with early-stage breast cancer. An additional component of the study was to analyze the data according to hormone receptor and HER2 status. Recently, Wildiers et al. (22) reviewed four adjuvant trials which had demonstrated the taxane-first regimens were favorable in terms of the relative drug dose intensity achieved. Also they mentioned larger non-randomized adjuvant studies for a series of 284 patients who first received three cycles of FEC followed by three cycles of docetaxel, the mean relative dose intensity was 91% for FEC and 76% for docetaxel, whereas in another series of 378 patients who received three cycles of docetaxel followed by four cycles of EC (epirubicin plus cyclophosphamide), a median docetaxel dose intensity of 100% was achieved. Therefore, they concluded such data suggest that the administration of a taxane first, followed by an anthracycline, may be preferable in line with the Norton–Simon hypothesis (23). In the JBCRG 01 study, the largest study to date to evaluate neoadjuvant chemotherapy in this patient population, the clinical and pathological responses

Table 5. Predictive variables for pCR before and following chemotherapy

Variables	Before treatment			After DOC			After FEC following DOC		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
Menopausal status: pre (versus post)	1.5	0.94–2.40	0.0923	1.52	0.94–2.47	0.0867	1.42	0.87–2.31	0.1575
Tumor size: ≥3 cm (versus <3 cm)	1.51	0.94–2.41	0.0881	1.45	0.90–2.34	0.1266	1.56	0.96–2.52	0.0724
ER: negative (versus positive)	0.58	0.32–1.03	0.0650	0.51	0.28–0.95	0.0331	0.58	0.32–1.05	0.0709
PgR: negative (versus positive)	0.66	0.34–1.28	0.2211	0.72	0.37–0.95	0.3408	0.65	0.33–1.27	0.2083
HER2: 3+ (versus <3+)	1.32	0.76–2.28	0.3251	1.41	0.80–2.47	0.2360	1.39	0.80–2.41	0.2445
Clinical response to DOC									
Response (versus no response)	—	—	—	0.64	0.38–1.07	0.0875	—	—	—
Clinical response to FEC following DOC									
Response (versus no response)	—	—	—	—	—	—	0.58	0.29–1.14	0.1160

CI, confidence interval; OR, odds ratio; pCR, pathologic complete response.

and safety of FEC followed by docetaxel were investigated (19). The eligibility criteria, treatment dose and distribution of patient characteristics (menopausal status, tumor stage, hormone receptor status and HER2 status) studied in the JBCRG 01 trial were similar to those investigated in the present JBCRG 03 study (19). The incidences of Grade 3/4 neutropenia and febrile neutropenia observed in the current study were similar to those reported in the JBCRG 01 trial (19). However, the rate of Grade 1/2 edema during docetaxel treatment was lower in the present study (33%) than in the JBCRG 01 study (41%), suggesting that docetaxel might be better tolerated when given up front than when administered after completion of prior chemotherapy. Further studies are warranted to assess quality of life and the incidence of edema in order to confirm the effect of administering docetaxel as the initial therapy.

Many different neoadjuvant chemotherapy schedules and dose regimens are used in clinical practice. The NSABP Protocol B-18 trial, which compared AC treatment before and after surgery, reported no difference in DFS between the two approaches (17). However, the rate of BCS was greater with neoadjuvant AC chemotherapy, and the prognosis of patients who obtained a pCR was also better with this treatment regimen (17). Several other regimens have been evaluated in an effort to increase the pCR rate. The addition of a taxane to an anthracycline-containing regimen has been shown to improve the pCR and clinical RRs (5,18). Furthermore, excellent results have been reported by the MD Anderson Cancer Center using a regimen of paclitaxel plus trastuzumab followed by FEC plus trastuzumab in patients with operable breast cancer and HER2 overexpression (24). However, few studies have evaluated initial taxane therapy followed by an anthracycline-containing regimen in this indication (24). Thus, it was decided to evaluate such a reverse regimen and to analyze the findings according to molecular subtypes. Importantly, the primary endpoint—pCR rate—achieved in the present study was 23% (95% CI, 16–31%), far exceeding our estimate of 12% (19). Even though the pCR rate here cannot be directly compared with the results from the JBCRG 01 trial (pCR rate: 12%, QpCR rate: 25%), the pCR rate from this study is a favorable result considering the similar patient characteristics in both trials (19).

The overall clinical RR of 79% was similar to that reported in the JBCRG 01 trial (74%) (19). Furthermore, the clinical RR following the initial docetaxel regimen was 64%, similar to the clinical response following the initial FEC regimen in the JBCRG 01 trial (61%) (19). The clinical RR following the initial docetaxel regimen, however, is lower in this study than those reported in other studies (71.7–85%) (25,26). It could be hypothesized that the clinical response might be influenced by the lower dose of docetaxel used in this study (75 mg/m^2) compared with the 100 mg/m^2 dose used in previous studies (25,26).

The rate of BCS observed in our study (79%) was similar to that reported in the JBCRG 01 trial (85%) (19). Unfortunately, the overall disease progression rate (5%) was not lowered by the use of docetaxel followed by FEC in this study, and was similar to that seen in the JBCRG 01 trial (6%) (19).

Although 7 of the 29 patients with triple-negative disease had disease progression during the initial docetaxel regimen, 14 of the 22 patients without disease progression (64%) achieved a QpCR. This QpCR rate is markedly higher compared with previous findings (27).

Our results indicate that if patients with triple-negative disease who experienced disease progression following initial docetaxel therapy were excluded, the pCR rate for this group of patients would have been higher. We thus compared the clinical and pathological characteristics between patients with triple-negative disease who experienced disease progression following the initial docetaxel regimen with those who did not have disease progression. However, no

significant differences in patient or tumor characteristics were seen between these patient groups. It was noted, however, that six of seven premenopausal patients (86%) and four of seven patients (57%) with solid-tubular carcinoma had disease progression following docetaxel therapy. Given the high incidence of disease progression among patients with triple-negative disease who had solid-tubular subtype tumors, this phenotype could be used in future studies to predict which patients are more likely to experience progressive disease following docetaxel therapy. Accordingly, the identification of patients with hormone receptor-positive and HER2-negative disease would also enable the selection of patients who are more likely to benefit from neoadjuvant chemotherapy. Thus, studying patients' molecular subtypes, and selecting appropriate chemotherapy regimens accordingly, has the potential to provide superior results to those of the JBCRG 03 trial.

Recently, it has been shown that basal-like breast cancer defined by five biomarkers [epidermal growth factor receptor (EGFR), cytokeratin 5/6 (CK5/6), ER, PgR and HER2 status] provides a more specific definition of basal-like breast cancer that predicts survival better than the triple-negative phenotype (27,28). In patients treated with anthracycline-based chemotherapy, tumors found to be positive for the basal markers corresponded to a cohort of patients with a significantly worse outcome (29). Thus in future trials, it may be beneficial to assess EGFR and CK5/6 status in patients with triple-negative disease to help predict patient survival.

Interestingly, the pCR rate (27%) following neoadjuvant chemotherapy in patients with HER2-negative breast cancer was higher in this study than in the JBCRG 01 study (14%), suggesting that this subpopulation may benefit from initial docetaxel treatment. Conversely, a lower QpCR rate was observed in HER2-positive patients (37%) in this study than in the JBCRG 01 trial (52.8%). This suggests that initial anthracyclines may be required for HER2-positive disease. A study by Buzdar et al. (24) reported that a high pCR rate of 60% was observed in patients with HER2-positive disease treated with the combination of paclitaxel plus trastuzumab followed by FEC plus trastuzumab, indicating that the HER2-positive population in the current study may have benefited further from concomitant trastuzumab therapy. These findings demonstrate the benefit of selecting the most effective chemotherapy regimen according to each patient's molecular subtype and initial response to neoadjuvant treatment.

One limitation of the study was that HER2-positive patients were not treated with trastuzumab, which has been shown to improve outcomes in patients with HER2-overexpressing breast cancer (24). Further studies investigating optimal treatment regimens for different molecular subtypes should include concurrent trastuzumab for patients with the HER2-positive phenotype.

In conclusion, docetaxel followed by FEC as neoadjuvant chemotherapy is a tolerable and effective regimen for patients with early-stage breast cancer. In addition, a high pCR rate made this regimen particularly promising in patients with triple-negative breast cancer. In the future, selection of a neoadjuvant chemotherapy regimen for operable breast cancer may be possible based on molecular subtype.

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Conflict of interest statement

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ORIGINAL ARTICLE

The efficacy and safety of gemcitabine plus paclitaxel combination first-line therapy for Japanese patients with metastatic breast cancer including triple-negative phenotype

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Abstract
Purpose Gemcitabine (GEM)–paclitaxel combination therapy has been confirmed as a standard therapy for metastatic/recurrent breast cancer (MBC) in Western countries. This study was conducted to assess the efficacy and safety of GEM–paclitaxel combination therapy in Japanese MBC patients.
Methods Patients were administered paclitaxel 175 mg/m^2 on day 1, and GEM 1,000 or 1,250 mg/m^2 on days 1 and 8 of 21-day cycle. The primary endpoint of this study was overall response rate; secondary endpoints were duration of response, time to progression, survival time and rate.
Results Paclitaxel 175 mg/m^2 plus GEM 1,250 mg/m^2 was determined as the recommended dose. A total of 56 patients received 506 cycles of treatment (median: 7.5 cycles) with a relative dose intensity of 79.6% for GEM and 85.8% for paclitaxel. The response rate was 44.6% (25/56 patients), median time to progression 8.6 months and median survival time 27.1 months. In triple-negative patients, the response rate was 35.7% (5/14 patients), and the median time to progression was 6.0 months. The most frequent grade ≥ 3 toxicities were neutropenia (82.1%), leukopenia (62.5%) and ALT increase (14.3%).
Conclusions This study confirmed the efficacy and safety of GEM–paclitaxel combination therapy in Japanese MBC patients.

Keywords Anthracycline-pretreated metastatic breast cancer · Triple negative · Gemcitabine · Paclitaxel · Phase I/II trial

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Introduction

Since 1990, the age-standardized breast cancer death rate has declined in many developed countries [1]; however, the mortality rate is still increasing in Japan [2].

Metastatic breast cancer remains an incurable disease despite progress in current treatment that has resulted in improved survival rates and quality of life. Chemotherapy is currently the treatment of choice for women with Her2/neu negative, endocrine-resistant MBC, or for women with extensive visceral localizations or life-threatening disease. The most used drugs are anthracyclines, taxanes, alkylating agents, anti-metabolites, and vinca-alkaloids. Anthracycline-based combinations remain the standard first-line treatment for MBC, but despite objective response rates in 50–60% of patients, median survival period does not exceed 2–3 years [3–5].

Combination first-line chemotherapy usually provides a higher response rate and longer progression-free survival compared with single-agent chemotherapy [6]. However, due to the availability of very effective second-line, third-line, or even fourth-line chemotherapy along with the recent development of effective molecular targeted therapy, very few trials show overall survival benefit for a combination strategy [7]. One of the exceptions is the combination of an anti-metabolite such as capecitabine or gemcitabine (GEM) with taxane.

Combination therapy with GEM (a nucleoside analog) and taxane offers specific advantages because of their distinct mechanisms of action with no overlapping toxicity, including lack of cardiotoxicity [8]. GEM has demonstrated synergistic effects with taxanes in preclinical tumor models [9, 10], and the two-drug combination of GEM–paclitaxel was studied in various other malignant conditions including non-small cell lung cancer [11], bladder [12], ovarian [13], and breast cancer [14]. In recent years, two phase III randomized clinical trials [15, 16] have shown the beneficial effects of combined therapy with GEM and taxane for the treatment of MBC. Further, an analysis of the global QoL endpoint favored the GEM–paclitaxel combination therapy over paclitaxel monotherapy despite an increase in myelosuppression [17]. Consequently, GEM in combination with paclitaxel has been approved in several countries including the United States and the European Union for the treatment of unresectable, locally recurrent, or metastatic BC in patients following anthracycline-based adjuvant/neoadjuvant chemotherapy.

Japanese patients are known to suffer from more bone marrow toxicity compared with patients from Western countries when treated with a paclitaxel containing regimen [18]. Although paclitaxel 175 mg/m^2 plus GEM 1,250 mg/m^2 has been established as a standard regimen for MBC in Western countries, the optimal dose, schedule, and sequence of administration still need to be determined in Japanese MBC patients. The present phase I/II clinical study was thus conducted using the same regimen to assess the efficacy and safety of GEM–paclitaxel combination therapy in Japanese MBC patients.

Patients and methods

Study design

This study was a multicenter, non-randomized, open-label, phase I/II study conducted in Japanese patients with metastatic/recurrent breast cancer (MBC) to assess the efficacy and safety of the GEM–paclitaxel combination therapy. This study consisted of two steps. At Step 1, the officially approved GEM dose for other cancers in Japan (GEM 1,000 mg/m^2) was administered with paclitaxel 175 mg/m^2 to the first group of patients as the initial dose of the study treatment. After confirmation of the safety at GEM 1,000 mg/m^2 plus paclitaxel 175 mg/m^2, an escalated dose of GEM 1,250 mg/m^2 plus paclitaxel 175 mg/m^2 was administered to a second group of patients. This specific dose was chosen because the combination of GEM 1,250 mg/m^2 and paclitaxel 175 mg/m^2 has been recommended in countries other than Japan according to the results from a phase III study [15].

Six patients were enrolled for each group in Step 1. Paclitaxel 175 mg/m^2 was administered intravenously over 3 h on day 1 and GEM 1,000 mg/m^2 was given intravenously over a 30-min infusion on days 1 and 8 in a 3-week cycle, 2 consecutive administration weeks followed by a 1-week rest period. If dose-limiting toxicity (DLT) occurred in less than 2 out of 6 patients at GEM 1,000 mg/m^2, the dose was increased to GEM 1,250 mg/m^2 and paclitaxel 175 mg/m^2, and then administered to a second group of patients.

In Step 2, an additional 50 patients were enrolled and evaluated for the efficacy and safety at the recommended dose determined in Step 1. Treatment was repeated every 21 days until disease progression, intolerable toxicity or patient withdrawal.

Patients

Female patients with histologically or cytologically confirmed MBC or inoperable locally advanced BC were enrolled in the study. All MBC patients had relapsed after receiving anthracycline-based chemotherapy regimen in a neo-adjuvant/adjuvant setting, but no prior chemotherapy for metastatic disease. Neo-adjuvant/adjuvant chemotherapy

including taxanes must have been completed more than 12 months before registering in this study. Other inclusion criteria were as follows: good performance status (ECOG) 0 or 1, at least one bidimensionally measurable lesion, adequate function of major organs [hemoglobin $\geq$ 9.0 g/dL, neutrophils $\geq$ 2,000/mm^3, platelets $\geq$ 100,000/mm^3, AST/ALT $\leq$ 2.5 times upper limit of normal (ULN), ALP $\leq$ 2.5 times ULN, $\leq$ 5.0 times ULN for patients with liver or bone metastases] and an estimated life expectancy of at least 12 weeks. Written informed consent was obtained from all patients enrolled in the study.

This study was conducted in compliance with the guideline of good clinical practice and the Declaration of Helsinki, and the study protocol was approved by the local institutional review boards. The Efficacy and Safety Evaluation Committee, an independent review board, was consulted if any efficacy or safety issues arose in the study.

Efficacy measures

The primary objective of this study was to confirm that the lower limit of the 95% confidence interval (CI) of the response rate at the recommended dose exceeded the threshold response rate of 25%. Tumor response was evaluated in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST 2000). Responder was defined as a patient who met either the complete response (CR) or partial response (PR) criteria for overall response assessment. CR or PR was confirmed at least 4 weeks after first observation of the response.

Secondary objectives included the median duration of response, time to progression, median survival time, and 1- and 2-year survival rates. The duration of response was the period from the day when the patient first satisfied either the CR or PR criteria to the day when the patient first met the criteria of progressive disease (PD). Time to progression (TTP) was defined as the period from the registration day to the time when any indication of disease progression (including increased size of tumor, identification of a new lesion, death, and aggravation of symptoms) was observed. Survival time was defined as the period from the date of registration to the date of death (regardless of the cause of death). Patients alive at the end of the follow-up period were treated as censored cases.

Safety measures

The safety evaluation included the type and incidence of adverse events. All adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 10.0; toxicities were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

DLT was defined as a toxicity occurring in cycle 1 that met one of the following criteria: neutropenia of $\geq$grade 3 with a fever of $\geq$38.0°C, thrombocytopenia of <25,000/mm^3 or thrombocytopenia with bleeding that required platelet transfusion(s), non-hematotoxicity of $\geq$Grade 3 (excluding nausea, vomiting, anorexia). A delay in the start of cycle 2 was also classified as a DLT if cycle 2 could not be started within 42 days after the initiation of cycle 1 due to study drug toxicity.

Statistics

All patients who received at least one dose of the study drug were included in the efficacy and safety analysis. The primary efficacy endpoint was response rate. A statistical test against the null hypothesis of "the response rate is less than 25%" was performed by obtaining an exact *P*-value based on the binomial distribution with a significance level of 2.5% (one-sided). The other efficacy endpoints of duration of response, time to progression (TTP), survival time and 1- and 2-year survival rates were estimated using the Kaplan–Meier method. Two-sided 95% CIs for all endpoints were obtained.

The sample size was determined by reference to the results of a global phase III study [15]. The expected response rate of the GEM–paclitaxel combination treatment and the threshold response rate were set at 45% and 25% respectively. Assuming that the true response rate is 45%, the number of 48 subjects is needed to achieve 80% power when the statistical test is applied based on the binomial distribution with a significance level of 2.5% (one-sided). As this was the first time of the GEM–paclitaxel combination treatment to Japanese patients with MBC, given adequate consideration for feasibility, it was necessary to treat at least 55 patients with the recommended dose to evaluate the safety profile.

Results

Patient disposition and characteristics

This study was carried out from June 2006 to August 2009 at 24 study centers in Japan. Sixty-two female patients were enrolled into this study. At Step 1, 12 patients were divided into two groups of 6 patients each and administered paclitaxel 175 mg/m^2 plus GEM 1,000 mg/m^2 or GEM 1,250 mg/m^2 to determine the recommended dose for Step 2. At Step 2, an additional 50 patients were enrolled at the recommended dose of GEM plus paclitaxel 175 mg/m^2.

The mean age was 58.2 years (range 51–63) in the GEM 1,000 mg/m^2 dose group and 54.4 years (range 30–73) in the GEM 1,250 mg/m^2 dose group. All 62 patients had a history of prior chemotherapy; 27 were anthracycline and taxane pretreated patients and 35 anthracycline pretreated patients. Fifty-five patients had metastases: 32 lung, 25 bone, 22 liver and 20 lymph nodes (Table 1).

Table 1 Baseline demographic and characteristics of patients

	G 1000 group	G 1250 group
Patient number (%)	6 (100.0)	56 (100.0)
Age: mean (SD)	58.2 (4.5)	54.4 (8.7)
Height (cm): mean (SD)	153.5 (8.0)	154.7 (6.2)
Body weight (kg): mean (SD)	57.2 (15.3)	55.8 (8.7)
PS (ECOG)		
0	4 (66.7)	50 (89.3)
1	2 (33.3)	6 (10.7)
Metastatic sites		
Patients without metastases	1 (16.7)	6 (10.7)
Patients with metastases	5 (83.3)	50 (89.3)
Lung	3 (50.0)	29 (51.8)
Bone	1 (16.7)	24 (42.9)
Liver	2 (33.3)	20 (35.7)
Brain	0 (0.0)	2 (3.6)
Lymph node	2 (33.3)	18 (32.1)
Skin	0 (0.0)	4 (7.1)
Other sites	1 (16.7)	14 (5.0)
Estrogen receptor status		
Positive	1 (16.7)	35 (62.5)
Negative	5 (83.3)	21 (37.5)
Progesterone receptor status		
Positive	1 (16.7)	26 (46.4)
Negative	5 (83.3)	30 (53.6)
Her2/neu expression status		
0	4 (66.7)	19 (33.9)
1+	2 (33.3)	22 (39.3)
2+	0 (0.0)	1 (1.8)
3+	0 (0.0)	9 (16.1)
Unknown	0 (0.0)	5 (8.9)
Prior therapy		
Surgical therapy	3 (50.0)	42 (75.0)
Chemotherapy	6 (100.0)	56 (100.0)
Radiotherapy	2 (33.3)	25 (44.6)
Hormonal therapy	1 (16.7)	33 (58.9)
Other	2 (33.3)	16 (28.6)
Prior chemotherapy		
Anthracycline plus taxane	2 (33.3)	25 (44.6)
Anthracycline	4 (66.7)	31 (55.4)

PS performance status, *ECOG* Eastern cooperative oncology group, *Her2* human epidermal growth factor receptor 2

Triple-negative patients were defined as those with negative estrogen receptor (ER) and progesterone receptor (PR) status and an Her2/neu status of 0 or 1+. Fourteen of the 56 patients in the GEM 1,250 mg/m^2 dose group met the triple-negative criteria.

Patients were also classified by hormone receptor subtype: 27 patients with ER+ or PR+ and HER2−, 7 patients with ER+ or PR+ and HER2+, 2 patients with ER− and PR− and HER2+.

Dose-limiting toxicity (DLT)

Two DLTs were observed: grade 3 ALT increase (1 patient at 1,000 mg/m^2) and grade 3 fatigue (1 patient at 1,250 mg/m^2). Therefore, GEM 1,250 mg/m^2 plus paclitaxel 175 mg/m^2 was determined as the recommended dose of this study.

Drug exposure

A total of 506 cycles were administered (median 7.5 cycles, range 1–37 cycles) at the GEM 1,250 mg/m^2 dose level. Relative dose intensities were 79.6% for GEM and 85.8% for paclitaxel.

Efficacy

The response rate was 44.6% at the GEM 1,250 mg/m^2 dose level, median duration of response was 7.9 months (95% CI: 5.6, 11.0), and the median TTP was 8.6 months (95% CI: 6.5, 10.3) (Table 2). The 1-year survival rate was 78.6% (95% CI: 67.8, 89.3). The 2-year survival rate was 58.9% (95% CI: 46.0, 71.8), with 30 out of 56 patients surviving at the time of the 2-year survival analysis. The median survival time was 27.1 months (95% CI: 22.9, incalculable) at the median follow-up time period of 24.8 months (Figs. 1, 2).

Among the 14 triple-negative patients, the response rate was 35.7% with 5 patients achieving PR. The median TTP in the triple-negative patients was 6.0 months (95% CI: 1.4, 7.3) compared with 9.6 months (95% CI: 7.4, 13.6) in the non-triple-negative patients (Table 2). The 27 patients with ER+ or PR+ and HER2− hormonal receptor subtype achieved a 59.3% response rate and a median TTP of 9.3 months (95% CI: 7.4, 15.4).

Safety

All 62 patients reported at least one adverse event, and hematological toxicity was commonly observed at the GEM 1,250 mg/m^2 dose level. The most common grade $\geq$ 3 drug-related adverse events were neutropenia

Table 2 Tumor response and time-to-event (RECIST criteria)

	N	Tumor response *n* (%)						Time to event median (months) (95% CI)	
		CR	PR	SD	PD	NE	RR (95% CI)	DOR	TTP
G 1250 group	56	0 (0.0%)	25 (44.6%)	14 (25.0%)	11 (19.6%)	3 (5.4%)	44.6% (31.3, 58.5)	7.9 (5.6, 11.0)	8.6 (6.5, 10.3)
Triple negative	14	0	5	4	5	0	35.7%	4.5 (2.8, 9.3)	6.0 (1.4, 7.3)
Non-triple negative	42	0	20	13	6	3	47.6%	8.2 (7.3, 13.2)	9.6 (7.4, 13.6)

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *NE* not evaluable, *RR* response rate, *DOR* duration of response, *TTP* time to progression, 95% CI: 95% confidence interval

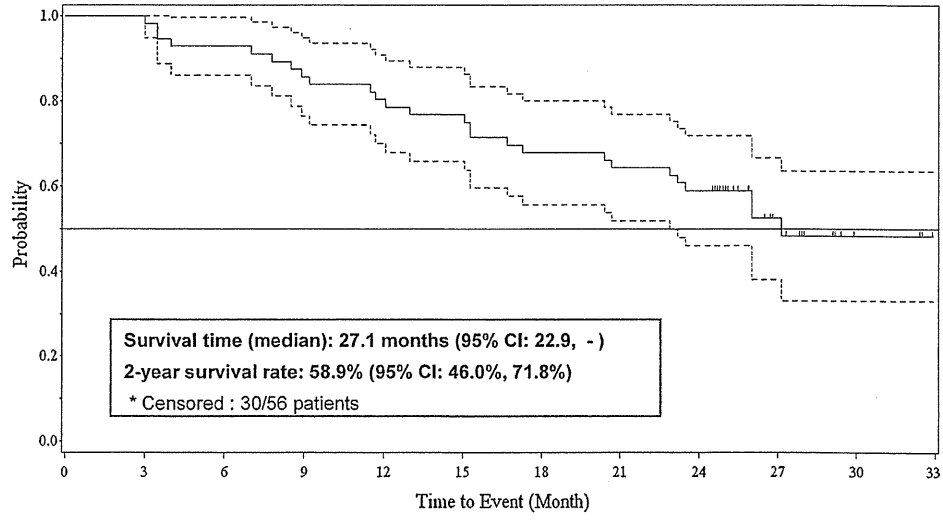

Fig. 1 Kaplan–Meier survival curve

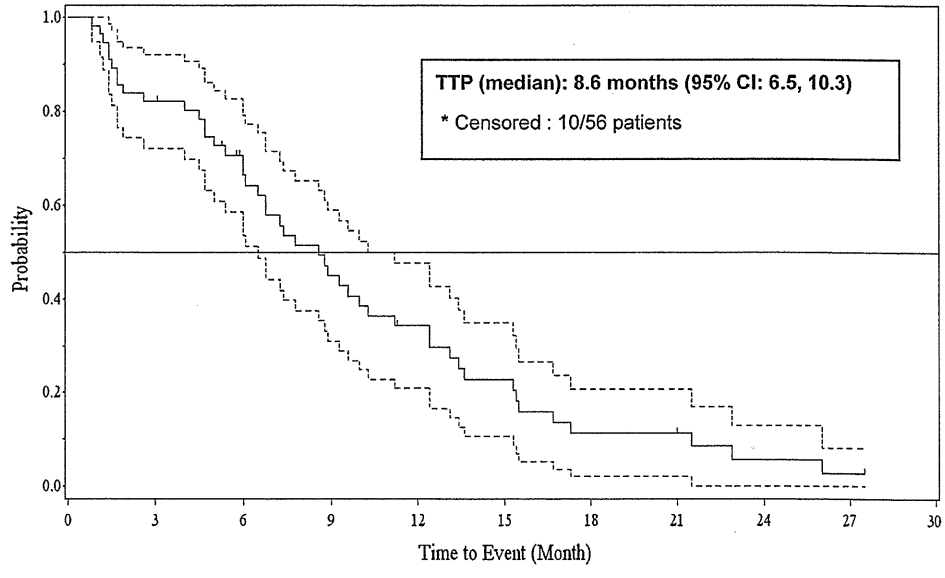

Fig. 2 Kaplan–Meier time to progression (*TTP*) curve

(82.1%), leukopenia (62.5%), lymphopenia and alanine transaminase (ALT) increase (14.3% each). The incidence of grade 3 non-hematological toxicity was low (Table 3). Fourteen of 56 patients (25.0%) reported peripheral neuropathy, but with no grade 3 or 4 toxicities. The incidence of neutropenia was 82.1%; however, no case of febrile neutropenia was reported. Prophylactic use of G-CSF was not allowed in this study, and only 10 patients (10/56, 17.9%) received G-CSF during the 2-year follow-up period. No patients required platelet transfusions.

Table 3 Adverse reactions (CTC grade 2, 3 or 4 toxicities)

Parameters	Toxicity grade[a] ($N = 56$)					
	Grade 2		Grade 3		Grade 4	
	n	%	*n*	%	*n*	%
Hematologic						
Neutrophil count decreased	7	12.5	18	32.1	28	50.0
White blood cell count decreased	12	21.4	30	53.6	5	8.9
Lymphocyte count decreased	18	32.1	4	7.1	3	5.4
ALT increased	24	42.9	7	12.5	0	0.0
Hemoglobin decreased	21	37.5	4	7.1	0	0.0
Platelet count decreased	8	14.3	5	8.9	0	0.0
AST increased	8	14.3	4	7.1	0	0.0
Red blood cell count decreased	13	23.2	3	5.4	0	0.0
GGT increased	3	5.4	2	3.6	0	0.0
Blood albumin decreased	4	7.1	0	0.0	0	0.0
Febrile neutropenia	0	0.0	0	0.0	0	0.0
Non-hematologic						
Alopecia	25	44.6	0	0.0	0	0.0
Malaise	9	16.1	1	1.8	0	0.0
Pain in extremity	9	16.1	1	1.8	0	0.0
Rash	9	16.1	0	0.0	0	0.0
Arthralgia	8	14.3	1	1.8	0	0.0
Peripheral neuropathy	7	12.5	0	0.0	0	0.0
Constipation	6	10.7	0	0.0	0	0.0
Diarrhea	4	7.1	2	3.6	0	0.0
Myalgia	4	7.1	1	1.8	0	0.0
Fever	4	7.1	1	1.8	0	0.0
Vomiting	4	7.1	0	0.0	0	0.0
Nausea	3	5.4	0	0.0	0	0.0
Anorexia	2	3.6	1	1.8	0	0.0

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* gamma glutamyltransferase, *ALP* alkaline phosphatase

[a] Toxicity was graded according to CTCAE v3.0

Discussion

This phase I/II, multicenter study was conducted to evaluate the efficacy and safety of GEM plus paclitaxel combination therapy in Japanese MBC patients who had received prior chemotherapy with anthracycline.

Selection of combination versus serial single chemotherapy in the metastatic setting has been debated. The concepts of non-overlapping resistance mechanisms and toxicity profile have been the guiding principle for modern combination chemotherapy such as used in lymphoma, certain leukemia, and testicular germ cell cancers, but the validity of this concept has not been consistently shown in MBC. In randomized trials where single-agent and combination chemotherapy for MBC were compared, only a few multidrug therapies (capecitabine plus docetaxel or GEM plus paclitaxel) were associated with improvement in overall survival [15, 19], possibly because effective first-line chemotherapy can mask the true efficacy of second or later-line chemotherapy. In a phase III study that compared the GEM–paclitaxel combination with paclitaxel monotherapy, randomized patients were required to have had a history of anthracycline containing chemotherapy, and thus fewer patients in the monotherapy arm responded to paclitaxel monotherapy. In that study, more than 90% of the patients had prior anthracycline therapy resulting in a lower response rate for paclitaxel monotherapy [15].

Although therapy with serial single agents is a reasonable and often preferred alternative to combination regimens, combination therapy may be a more appropriate first-line choice, especially for symptomatic patients or those with rapidly progressive visceral metastases because of the greater likelihood of an objective response. Furthermore, analysis of the global QoL endpoint from a phase III study favored the GEM–paclitaxel combination therapy over paclitaxel monotherapy. The benefits of this combination as shown by QoL differences and mean global QoL scores rated by the Rotterdam Symptom Checklist (RSCL) indicated significant improvement of patients in GEM–paclitaxel combination arm versus paclitaxel monotherapy arm [17]. Capecitabine and docetaxel share hand–foot syndrome as an overlapping toxicity. Retrospective analysis of 1,000 Japanese breast cancer patients showed that the incidence of grade 2 or higher hand–foot syndrome with docetaxel was 16%, which increased to 40% with the docetaxel–capecitabine combination [20]. Results from another phase III study have also suggested that GEM may be a better option than capecitabine in combination with docetaxel for the treatment of advanced BC [16].

The present study also revealed that GEM–paclitaxel therapy was well tolerated in Japanese patients with 19 of 56 patients able to continue the study treatment for more than 10 cycles, adverse events that occurred in this study were manageable with appropriate treatment. The most common clinically significant adverse events encountered with this combination therapy were related to myelosuppresion such as neutropenia (82.1%), leukopenia (62.5%) and lymphocytopenia (12.5%). However, patients were able to continue the treatment over 506 cycles administered in 56 patients (median 7.5 treatment cycles, range 1–37). Grade 3 ALT increases were reported in 7 patients (12.5%); however, the study protocol allowed patients with liver metastases to enroll. At study entry, 20 patients had liver metastases and elevated liver enzymes associated with symptomatic aggravation. Results obtained in the present study were similar to those obtained in an earlier randomized phase III global trial [15] which demonstrated significant benefit of the GEM–paclitaxel combination therapy

over paclitaxel alone in the treatment of advanced breast cancer. Indeed, RR (44.6%) as well as TTP (8.6 months) observed in the present study were numerically better than the results (41.4% and 6.1 months, respectively) reported in the global trial. The 1- and 2-year survival rates observed in the present Japanese study (78.6 and 58.9%, respectively) were also greater than the survival rates (71 and 41%) observed in the global trial. The median survival time in this study was 27.1 months (95% CI: 22.9, incalculable) with more than half the patients were surviving after the 2-year follow-up period. This clearly demonstrates the beneficial effect of the GEM–paclitaxel combination on survival.

The response rate in the present study was similar to the other GEM–paclitaxel combination phase II first-line study [21]. Response rates for MBC in that study were 40–50%, while Delfino et al. reported a higher response rate of 66.7% (30/45 patients, 10 CR and 20 PR) in their phase II study [22]. This might be due to differences in patients' background in that more than half of the patients (53.3%) had no history of prior chemotherapy in the Delfino study.

Another important observation made in the present study relates to the effects of the GEM–paclitaxel combination therapy on patients with triple-negative breast cancer (TNBC). It is estimated that over 1 million women worldwide will be diagnosed annually for breast cancer and that 15% of them are likely to be classified as patients with TNBC [23, 24], with 30% of these patients developing metastatic disease [25]. TNBC usually exhibits an aggressive clinical course unlike hormone receptor-positive breast cancer and previously had not been a candidate for target therapy such as HER-2-positive breast cancer. Therefore, patients with TNBC are more likely to develop distant metastasis in locations like the brain earlier than non-TNBC patients, and have shorter overall survival [26]. Lin et al. reported that close to 50% of TNBC develop brain metastasis, and one-third of which were at first site of recurrence [27].

Until recently, the subset of breast cancer patients with triple-negative disease lacked a distinct therapeutic approach, despite this accounting for 15% of breast cancer patients. For patients with TNBC, anti-estrogen therapy and HER2 targeted agents are not useful options, and strategies utilizing both standard cytotoxic agents and novel targeted therapy have evolved [28]. A recent randomized phase II study on the efficacy of a PARP (poly ADP ribose polymerase) inhibitor in combination with GEM and carboplatin in patients with TNBC has shown that the median PFS was 6.9 months [29]. In comparison, combination therapy with GEM–paclitaxel at the recommended dose level (GEM 1,250 mg/m^2) resulted in a 7.9 month median duration of response for the 25 responding patients in this study. It was also found that TTP in triple-negative patients was 6.0 months versus 9.6 months in non-triple-negative patients.

Hormone receptor-positive patients have longer progression-free survival compared with triple-negative (TN) type patients. In the E2100 trial, where paclitaxel was compared with the paclitaxel plus bevacizumab, the PFS of TN patients was shorter compared with hormone receptor-positive patients in the paclitaxel arm (5.3 vs. 10.6 months) [30]. Although cross-trial comparison is limited by many biases and our study used tri-weekly paclitaxel, our results also showed a similar trend (TN 6.0 vs. non-TN 9.6 months). Since weekly administration of paclitaxel has an advantage over tri-weekly administration [31], the combination of GEM with weekly paclitaxel, possibly incorporating bevacizumab might result in better tumor control. This is being tested in ongoing clinical trial [32].

Even though the efficacy of combination therapy in the treatment of TNBC needs to be further studied, the results from the present study are in agreement with similar observations made in the earlier global trial [15] and indicate that GEM–paclitaxel combination therapy would be effective and well tolerated in Japanese patients with MBC.

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Conflict of interest statement The following authors disclose relationships with Eli Lilly Japan: TF and JF are employed by Eli Lilly and MT contributed in an advisory role.

Appendix

The following institutions participated in this study.

Sapporo Breast Surgical Clinic, Iwate University Hospital, Gunma Prefectural Cancer Center, Saitama Red Cross Hospital, Chiba Cancer Center, Kameda General Hospital, Tokyo Metropolitan Cancer and Infectious Disease Center Komagome Hospital, St Luke's International Hospital, Tokai University Hospital, Seirei Hamamatsu General Hospital, Aichi Cancer Center Hospital, Osaka National Hospital, Osaka Medical Center for Cancer and Vascular Diseases, Osaka Breast Clinic, Kinki University Hospital, Sakai Municipal Hospital, Kure Medical Center and Chugoku Cancer Center, Shikoku Cancer Center, Fukuoka University Hospital, Kyushu Cancer Center, Kumamoto Municipal Hospital, Breastpia Namba Hospital, and Sagara Hospital.

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Ki67 index changes, pathological response and clinical benefits in primary breast cancer patients treated with 24 weeks of aromatase inhibition

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Aromatase inhibitor shows efficacy for hormone receptor positive postmenopausal breast cancer. We evaluated the activity of 24 weeks of aromatase inhibition with exemestane for primary breast cancer in a neoadjuvant setting. Patients with stage II/IIIA invasive breast cancer with estrogen receptor (ER) and/or progesterone receptor (PgR)-positive status were eligible. Primary endpoints were objective response rate (ORR) and safety. A steroidal aromatase inhibitor exemestane of 25 mg/day was administered for 16 weeks with an 8-week extension. Secondary endpoints were rates of breast-conserving surgery (BCS), and change of Ki67 index and ER/PgR expression in central laboratory analyses. Between March 2006 and December 2007, 116 patients were enrolled. Among those, 102 patients completed 24 weeks of administration. The ORR was 47% (55/116) at Week 16 and 51% (59/116) at Week 24, respectively. No serious toxicity was seen. ORR was associated with ER Allred scores but not with PgR scores. The significant reduction in Ki67 index was confirmed. No progression was experienced in tumors with less than 15% Ki67 index. Pathological response was observed in 28 (30%) of 94 evaluated cases. No statistical correlation between pre-treatment Ki67 index and pathological response was detected; however, a trend of correlation was found between the post-treatment preoperative endocrine prognostic index (PEPI), a prognostic score and the pathological response. At diagnosis, 59 patients (51%) would have required mastectomy but 40 patients were converted to BCS, showing an increase in the rate of BCS (77%). The 24-week aromatase inhibition provided preferable clinical benefits with significant reduction in Ki67 index. More precise mechanisms of the response need to be investigated. (*Cancer Sci* 2011; 102: 858–865)

Many studies of neoadjuvant chemotherapy for breast cancer have been conducted. These studies have revealed that neoadjuvant chemotherapy allows more women to undergo breast-conserving surgery (BCS) rather than total mastectomy, and prolongs the survival of patients who achieved pathological complete response (pCR).[1–3] However, it has been described that neoadjuvant chemotherapy has a limited effect in hormone receptor-positive patients in terms of pCR rates, and raises safety concerns for elderly patients.[4–7] Therefore, as a treatment strategy, the efficacy and safety of neoadjuvant hormone therapy using aromatase inhibitors (AI) is being assessed in several trials in postmenopausal breast cancer patients.[8–11]

In a phase II randomized study in which neoadjuvant hormone therapy and neoadjuvant chemotherapy were compared in hormone receptor-positive patients, no significant difference in the clinical response rate was observed between these two groups. Notably, the rate of BCS tended to be higher, and the incidence of adverse events was generally lower in the neoadjuvant hormone therapy group than in the neoadjuvant chemotherapy group.[12] These results suggest the benefit of neoadjuvant hormone therapy in hormone-sensitive postmenopausal breast cancer patients.[13] Therefore, it seems that neoadjuvant hormone therapy offers an alternative to neoadjuvant chemotherapy.

However, there are some concerns surrounding the use of neoadjuvant hormone therapy that need to be addressed. First, tumor regression is slower with neoadjuvant hormone therapy than with chemotherapy. In fact, a study investigating the response rate to 6-month neoadjuvant hormone therapy using exemestane reported that the objective response rate (ORR: complete response [CR] + partial response [PR]) continued to increase even after 4 months of treatment.[14] Another concern is that there is no established index for evaluating the efficacy of neoadjuvant hormone therapy. In neoadjuvant chemotherapy, the pCR rate can be used as a surrogate marker for the prognosis of patients.[2] However, it has been reported that, in estrogen receptor (ER)-positive patients, the proportion of patients who achieved a pCR was not significantly correlated with overall survival (OS) or disease-free survival (DFS).[15] In addition, several Phase II studies of neoadjuvant hormone therapy reported that pCR rates were from 0 to about 3%, which were remarkably lower than those expected from the benefit observed in adjuvant hormone therapy.[8,11,12] Therefore, in hormone receptor-positive breast cancer patients, pCR is unlikely to be a useful marker for assessing efficacy or prognosis. A possible alternative marker for neoadjuvant hormone therapy is the percentage of MIB1/Ki67-positive cells (MIB-1/Ki67 labeling index), a cell proliferative index. The Ki67 index after neoadjuvant hormone therapy was shown to correlate with the recurrence rate.[16,17] However, the usefulness of the Ki67 index has not been fully evaluated.

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