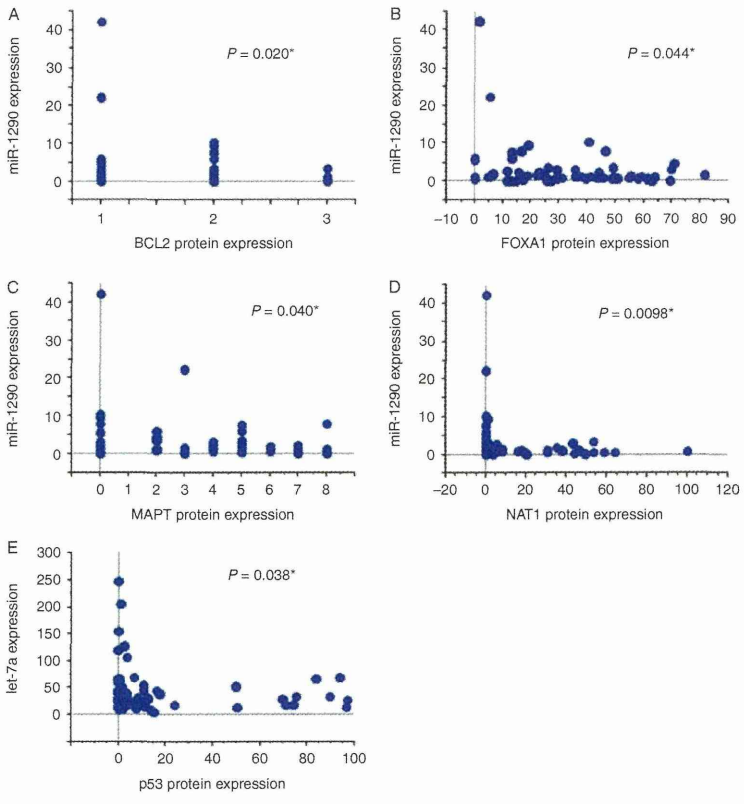

Figure 1

miR-1290 expression is inversely correlated with expressions of BCL2, FOXA1, MAPT, and NAT1. Scatter plots show inverse correlations between miR-1290 and BCL2 (A), FOXA1 (B), MAPT (C), and NAT1 (D) protein expression in breast cancer tissue ($P=0.020$, $P=0.044$, $P=0.040$, and $P=0.0098$ respectively). (E) let-7 expression is inversely correlated with P53 protein expression in breast cancer tissue ($P=0.038$).

Discussion

In this study, we have shown distinct expression patterns of miRNAs and mRNAs in luminal A and luminal B subtypes in ER-positive breast cancer. We demonstrated that miR-1290 and its potential target genes, *FOXA1* and *NAT1*, might be associated with characteristics of ER-positive disease. miR-1290 expression was strongly downregulated in ER^{high} $Ki67^{low}$ tumors and was positively correlated with tumor grade. Although the role of miR-1290 has not been analyzed as yet, it was reported that 36 miRNAs, including miR-1290, were circulating at increased levels in patients with renal cell carcinoma and were overexpressed in corresponding renal cell carcinoma tissue (Wulfken *et al.* 2011). It was also reported that six miRNAs, including miR-1290, were upregulated in drug-sensitive cells following Y-Box protein 1 inhibition, but no differences in miRNA expression could be detected in multidrug-resistant gastric carcinoma cells (Belian *et al.* 2010).

FOXA1, a forkhead family transcription factor, has been reported to be expressed predominantly in luminal A breast cancer with favorable prognosis (Badve *et al.* 2007, Mehta *et al.* 2012). Hurtado *et al.* recently reported that FOXA1 creates an open conformation at ER-binding sites and that ER can bind and activate target gene expression in the presence of estrogen. Thus, FOXA1 is a key determinant of ER function and endocrine response in breast cancer (Hurtado *et al.* 2011). They also reported that the differential ER-binding program observed in tumors from patients with poor outcome is due to the FOXA1-mediated reprogramming of ER binding (Ross-Innes *et al.* 2012). We demonstrated that FOXA1 expression is much higher in ER^{high} $Ki67^{low}$ tumors than in ER^{low} $Ki67^{high}$ tumors and that expression levels of FOXA1 were strongly and positively correlated with expression levels of ER and

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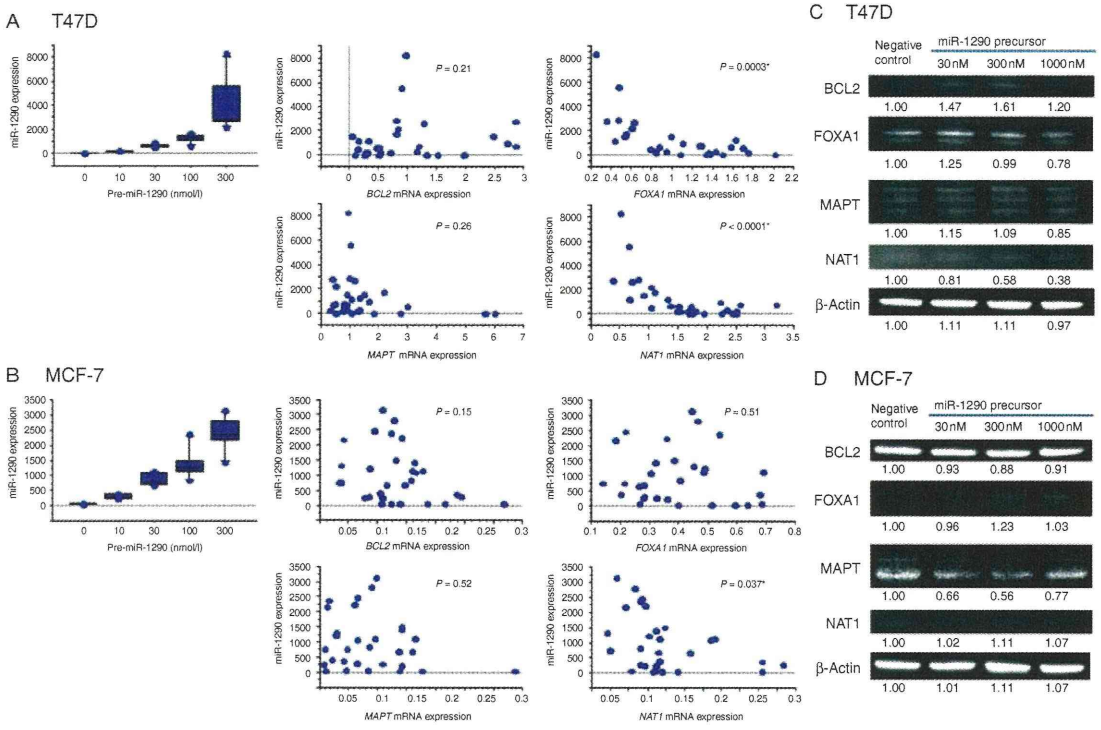

Figure 2

Gene expressions of miR-1290 putative targets in T47D and MCF-7 cells transfected with miR-1290. (A) T47D cells were transfected with either control miRNA (300 nmol/l) or pre-miR-1290 precursor at 10–300 nmol/l and incubated for 24 h. Expression levels of miR-1290 and mRNA levels of BCL2, FOXA1, MAPT, and NAT1 were measured by quantitative RT-PCR. Scatter plots show inverse correlation between miR-1290 expression and *FOXA1* and *NAT1* mRNA expression ($P=0.0003$ and $P<0.0001$ respectively). (B) MCF-7 cells were transfected with either control miRNA (300 nmol/l) or pre-miR-1290 precursor at 10–300 nmol/l and incubated for 36 h. Expression levels of miR-1290 and mRNA levels of BCL2, FOXA1, MAPT, and NAT1 were measured by quantitative RT-PCR. Scatter plots show inverse correlation between miR-1290 expression and *NAT1* mRNA expression ($P=0.037$). (C) T47D cells were transfected with either control miRNA (300 nmol/l) or pre-miR-1290 precursor at 30–1000 nmol/l and incubated for 48 h. Protein expression of BCL2, FOXA1, MAPT, and NAT1 was assayed by western blot analysis. The number below the band represents the mean value from densitometry reading, relative to the negative control, which was set at 1.00. Representative results from one of the three experiments are shown. (D) MCF-7 cells were transfected with either control miRNA (300 nmol/l) or pre-miR-1290 precursor at 30–1000 nmol/l and incubated for 48 h. Protein expression of BCL2, FOXA1, MAPT, and NAT1 was assayed by western blot analysis. The number below the band represents the mean value from densitometry reading, relative to the negative control, which was set at 1.00. Representative results from one of the three experiments are shown.

PgR and negatively associated with tumor grade in ER-positive breast cancer. Moreover, introduction of miR-1290 into estrogen-dependent breast cancer cells reduced FOXA1 expression. Because FOXA1 is a putative target of miR-1290 according to *in silico* analysis, we suggest that miR-1290 is a key factor for regulating FOXA1, which is associated with characteristics of ER-positive breast cancer.

Arylamine NATs, known as drug- and carcinogen-metabolizing enzymes, transfer an acetyl group from acetyl coenzyme A to arylamines (Sim *et al.* 2008). Several studies have shown higher mRNA and protein expression of NAT1 in ER-positive breast cancer compared with the expression in ER-negative disease (Perou *et al.* 2000, Adam *et al.* 2003, Tozlu *et al.* 2006, Wakefield *et al.* 2008). Moreover, it was reported that high expression of NAT1 was correlated with better outcome in ER-positive breast cancer (Bieche *et al.* 2004, Dolled-Filhart *et al.* 2006). Our results demonstrated that *NAT1* mRNA expression was much higher in ER^{high} $Ki67^{low}$ tumors than in ER^{low} $Ki67^{high}$ tumors by microarray analyses and that NAT1 protein expression by IHC showed positive correlation with expression levels of ER and PgR and negative correlation with expression levels of Ki67, tumor grade, and tumor size. In addition, introduction of miR-1290 into estrogen-dependent breast cancer cells strongly

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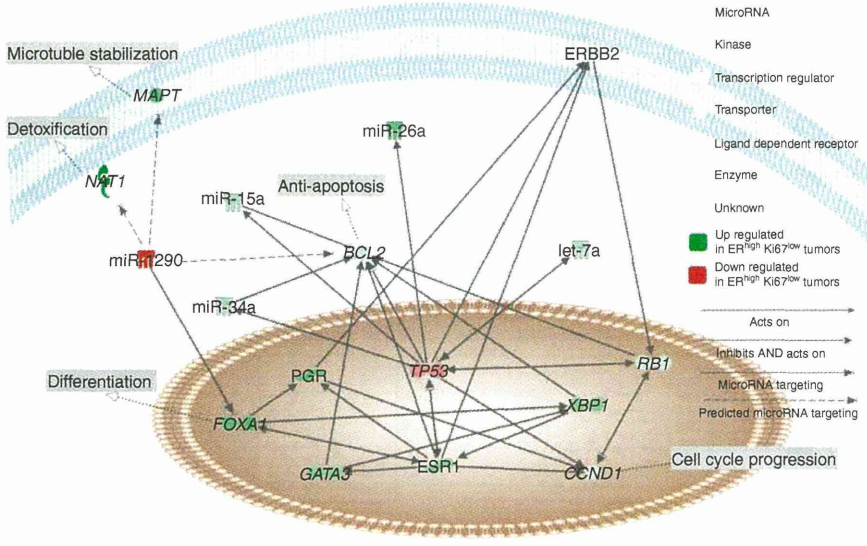

Figure 3

Interaction between miRNAs and putative target proteins that might be associated with characteristics of ER-positive breast cancer. Pathway analyses show five miRNAs (let-7a, miR-15a, miR-26a, miR-34a, and miR-1290) and nine target genes (*BCL2*, *CCND1*, *FOXA1*, *GATA3*, *MAPT*, *NAT1*, *RB1*, *TP53*, and *XBP1*) that were picked up in our present analyses. These proteins and their pathways have diverse cellular functions, such as differentiation, detoxification, anti-apoptosis, cell cycle progression, and microtubule stabilization.

reduced NAT1 expression. Because NAT1, as well as FOXA1, is a putative target of miR-1290 according to *in silico* analysis, it is possible that miR-1290 also regulates NAT1, which will be associated with characteristics of ER-positive breast cancer.

BCL2 and MAPT are also potential targets of miR-1290 according to *in silico* analysis. BCL2 is an anti-apoptotic protein that has an anti-proliferative effect influencing cell cycle entry (Zinkel *et al.* 2006). *BCL2* is an ER-induced gene, and its protein expression assessed by IHC has been shown to be a favorable prognostic marker in breast cancer (Callagy *et al.* 2006, Dawson *et al.* 2010). Our results also showed that expression levels of BCL2 were strongly and positively correlated with expression levels of ER and PgR in ER-positive breast cancer. It was recently reported that miR-195, miR-24-2, and miR-365-2 act as negative regulators of BCL2 through direct binding to their respective binding sites in the 3′-UTR of human *BCL2* gene (Singh & Saini 2012).

MAPT binds to both the outer and the inner surfaces of microtubules, leading to tubulin assembly and microtubule stabilization. As taxanes also bind to the inner surface of microtubules, MAPT might be considered to obstruct the function of these drugs. Most of the studies reported that MAPT expression has prognostic value, with high expression associated with favorable patient outcome. However, at the present time, there are few studies indicating that MAPT is a predictive marker for taxane-based chemotherapy (Baquero *et al.* 2011, Smoter *et al.* 2011). We demonstrated that expression levels of MAPT showed positive correlation with expression levels of ER and PgR and negative correlation with expression levels of Ki67, tumor grade, and tumor size in ER-positive breast cancer. Because miR-1290 did not decrease BCL2 or MAPT protein expression in ER-positive breast cancer cells in our analysis, BCL2 and MAPT might be regulated by other mechanisms.

Interaction between miRNAs and putative target proteins that might be associated with characteristics of ER-positive breast cancer is shown in Fig. 3, which was created by Ingenuity systems Pathway Analysis (http://www.ingenuity.com/index.html) and referring to previous reports (Gomez *et al.* 2007, Badve & Nakshatri 2009, Clarke *et al.* 2009, O'Day & Lal 2010).

Finally, our results indicated that let-7a was strongly upregulated in ER^{high} $Ki67^{low}$ tumors and that expression levels of p53, one of the let-7a targets, was inversely correlated with let-7a expression in ER-positive breast cancer. The let-7 miRNA family is a group of tumor suppressing miRNAs that can inhibit both tumorigenesis

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and metastasis (Zhang *et al.* 2010). It was recently reported that let-7 family miRNAs, especially let-7a, let-7b, and let-7i, were downregulated in breast cancer tissue compared with normal tissue and that let-7 miRNAs induced apoptosis in MCF-7 cells (Zhao *et al.* 2011). Thus, let-7 might have a role in ER-positive breast cancer.

In conclusion, this study indicates for the first time that miR-1290 and its potential targets, NAT1 and FOXA1, are strongly downregulated in ER^{high} $Ki67^{low}$ tumors and are associated with characteristics of ER-positive breast cancer. miR-1290 could be a novel therapeutic target in ER-positive breast cancer.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/ERC-12-0207.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
Y Endo designed the study, executed miRNA and mRNA expression profiling, target prediction and target validation, carried out immunostaining and western blotting, and drafted the manuscript. T Toyama, N Yoshimoto, M Iwasa, and T Asano provided tissue samples. S Takahashi assessed the immunostaining and western blotting. Y Fujii participated in its design and coordination. H Yamashita conceived of the study and participated in its design, coordination, and manuscript writing. All authors read and approved the final manuscript.

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CLINICAL TRIAL

Randomized trial of preoperative docetaxel with or without capecitabine after 4 cycles of 5-fluorouracil–epirubicin–cyclophosphamide (FEC) in early-stage breast cancer: exploratory analyses identify Ki67 as a predictive biomarker for response to neoadjuvant chemotherapy

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Abstract This randomized, multicenter study compared the efficacy of docetaxel with or without capecitabine following fluorouracil/epirubicin/cyclophosphamide (FEC) therapy in operable breast cancer and investigated the role of Ki67 as a predictive biomarker. Patients were randomized to 4 cycles of docetaxel/capecitabine (docetaxel: 75 mg/m^2 on day 1; capecitabine: 1,650 mg/m^2 on days 1–14 every 3 weeks) or docetaxel alone (75 mg/m^2 on day 1 every 3 weeks) after completion of 4 cycles of FEC (5-fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2 and cyclophosphamide 500 mg/m^2 on day 1 every 3 weeks). The primary endpoint was the pathological complete response (pCR) rate. Predictive factor analysis was conducted using clinicopathological markers, including hormone receptors and Ki67 labeling index (Ki67LI). A total of 477 patients were randomized; the overall response in the docetaxel/capecitabine and docetaxel groups was 88.3 and 87.4 %, respectively. There were no significant differences in the pCR rate (docetaxel/capecitabine: 23 %; docetaxel: 24 %; $p = 0.748$), disease-free survival, or overall survival. However, patients with mid-range Ki67LI (10–20 %) showed a trend towards improved pCR rate with docetaxel/capecitabine compared to docetaxel alone. Furthermore, multivariate logistic regression analysis showed pre-treatment Ki67LI (odds ratio 1.031; 95 % CI 1.014–1.048; $p = 0.0004$) to be a significant predictor of pCR in this neoadjuvant treatment setting. Docetaxel/capecitabine

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(after 4 cycles of FEC) did not generate significant improvement in pCR compared to docetaxel alone. However, exploratory analyses suggested that assessment of pre-treatment Ki67LI may be a useful tool in the identification of responders to preoperative docetaxel/capecitabine in early-stage breast cancer.

Keywords Breast cancer · Neoadjuvant chemotherapy · Ki67 · Capecitabine · Pathological complete response · Docetaxel

Introduction

Neoadjuvant chemotherapy has become increasingly significant in the treatment of operable early-stage breast cancer, with the advantage of the potential to downgrade tumors and increase the rate of breast conserving surgery (BCS) in patients that may have otherwise required a mastectomy [1]. Results from the National Surgical Adjuvant Breast and Bowel Project (NSABP) protocol B-18 trial demonstrated an increased likelihood in BCS in breast cancer patients treated with a neoadjuvant anthracycline-based regimen [1]. Although the B-18 trial did not demonstrate a survival advantage in patients treated with preoperative chemotherapy, it established pathological complete response (pCR) as a prognostic marker for disease-free survival (DFS). Indeed, pCR after neoadjuvant chemotherapy is considered a marker for favorable prognosis in breast cancer patients [2].

As such, clinical and molecular biomarkers capable of predicting pCR have been assessed following neoadjuvant treatment in breast cancer patients [3, 4]. In particular, the proliferation marker Ki67 has been reported to have predictive and prognostic value in patients with invasive breast cancer who received a range of neoadjuvant chemotherapy regimens, including anthracycline-based regimen without taxanes and anthracycline and taxane-based protocols [5].

While neoadjuvant treatment with anthracycline-based regimens is highly effective in the treatment of breast cancer, the sequential addition of a taxane to an anthracycline-based neoadjuvant regimen has been demonstrated to induce additive efficacy. In the NSABP B-27 trial, the sequential addition of docetaxel after doxorubicin and cyclophosphamide (AC) therapy doubled the rate of pCR, increased clinical response and increased the proportion of negative axillary nodes in early breast cancer patients [6]. In addition, 5-fluorouracil–epirubicin and cyclophosphamide (FEC) followed by docetaxel as neoadjuvant chemotherapy in the Japan Breast Cancer Research Group (JBCRG) 01 trial resulted in a pCR rate of 16 % with BCS possible for 85 % of the patients assessed [7].

In addition to inducing increased efficacy with anthracyclines, docetaxel has demonstrated significant synergy with the oral prodrug capecitabine [8]. Capecitabine is converted to 5-fluorouracil in a three-step process catalyzed by thymidine phosphorylase (TP) [9] and exhibits tumor specificity by exploiting the significantly higher activity of TP in tumor tissue in comparison to healthy tissue [8, 9]. Docetaxel has been demonstrated to upregulate TP expression in tumor tissues, possibly accounting for the synergistic effect observed with capecitabine [8]. Clinical studies have shown that single-agent capecitabine was an active and tolerable treatment for metastatic breast cancer (MBC) with disease progression during and after anthracycline and

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taxane therapy, achieving response rates of 20–29 % and a median survival in excess of 1 year [10, 11].

On the basis of these findings, the docetaxel/capecitabine regimen has been demonstrated to be well tolerated and effective for neoadjuvant treatment of stage II/III or locally advanced breast cancer [12–14]. Another study by O'Shaugnessy and colleagues also demonstrated a superior clinical response and survival outcome when the docetaxel/capecitabine regimen was compared with docetaxel alone in women with anthracycline-pretreated MBC [15]. However, these studies [12–15] did not undertake analyses to identify the tumor characteristics that define patients likely to respond to neoadjuvant docetaxel/capecitabine treatment.

Our randomized trial compared the efficacy of preoperative FEC followed by docetaxel with or without capecitabine in patients with early-stage breast cancer and assessed biomarkers that may be used to identify responders, in order to establish individualized treatment regimens.

Patients and methods

Study design

This multicenter, randomized, open study compared the efficacy of 4 cycles of FEC followed by 4 cycles of docetaxel and capecitabine or 4 cycles of docetaxel alone as neoadjuvant chemotherapy in patients with operable breast cancer. The study was approved by the Institutional Review Board of the Organisation of Oncology and Translational Research and conducted according to the Declaration of Helsinki. The primary endpoint was the pCR rate; secondary endpoints included toxicity, clinical response, frequency of breast and axillary lymph node conservation surgery, DFS, and overall survival (OS).

Patient eligibility

Women (20–70 years) with histologically confirmed operable invasive breast adenocarcinoma (T1C-3, N0, M0 (>1 cm)/T1-3, N1, M0) were eligible. In women without clinically suspicious axillary adenopathy, the primary breast tumor had to be >1 cm in diameter; patients with clinically suspicious axillary adenopathy could present with a primary tumor of any size (in accordance with cancer staging as per the American Joint Committee on Cancer).

Inclusion criteria were as follows: no prior treatment for breast cancer, Eastern Cooperative Oncology Group performance status of 0–1, white blood cell count >4,000–12,000 mm^3 or neutrophil count >2,000 mm^3, platelets >100,000 mm^3, hemoglobin >9.5 g/dL, bilirubin <1.25× institutional upper limit of normal (ULN), creatinine <1.5× institutional ULN, creatinine clearance >30 mL/min, aspartate aminotransferase and alanine aminotransferase <1.5× institutional ULN, a normal electrocardiogram for cardiac function, and left ventricular ejection fraction of >60 %.

Exclusion criteria included uncontrolled medical conditions, significant interstitial pneumonia or pulmonary fibrosis, suspected of infection with fever, symptomatic varicella, required treatment for pleural or pericardial effusions, severe edema, severe peripheral neuropathy, required steroid pre-treatment, severe psychiatric disorders, inflammatory breast cancer, bilateral cancer (if both tumors were within the inclusion criteria, bilateral cancer was not excluded), and a history of other malignancies within the last 5 years (except for adequately treated non-melanoma skin cancer or carcinoma in situ of the cervix).

Study treatment

Patients were scheduled to receive 4 cycles of intravenous FEC (5-fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2, cyclophosphamide 500 mg/m^2) on day 1 every 3 weeks. Patients who completed 4 FEC cycles were randomly assigned to receive either 4 cycles of docetaxel (75 mg/m^2, on day 1) plus capecitabine (825 mg/m^2 twice daily on days 1–14) or 4 cycles of docetaxel alone (75 mg/m^2, on day 1) every 3 weeks. For patients with a creatinine clearance of 30–50 mL/min, the initial dose of capecitabine was reduced to 75 % of the planned dose. Patients with disease progression while on FEC were excluded from randomization. A maximum 25 % dose reduction and 3-week dose delay were permitted for adverse events. Whereas a 75 % dose level was used as the initial dose for patients with low creatinine clearance, a further 25 % dose reduction was permitted for adverse events. Treatment prior to docetaxel comprised dexamethasone (8 mg oral; administered the morning and night before docetaxel). In addition, dexamethasone (10 mg intravenous) was administered 30 min before docetaxel. If a patient missed the 8 mg oral dexamethasone, the 10 mg intravenous dose was still administered and docetaxel administration occurred as planned. Primary surgery was undertaken within 3–6 weeks of neoadjuvant chemotherapy completion. Supportive care and postoperative endocrine or radiation therapy were administered at the investigator's discretion. No patients received trastuzumab before surgery, as it was not approved in Japan at the time of the study.

Study assessments

Pre-enrolment assessments included medical history, physical examination, blood chemistry, bilateral mammogram, bone and computed tomography scans. Initial diagnosis of invasive adenocarcinoma was made by core needle biopsy.

Estrogen receptor (ER) and progesterone receptor (PgR) status were confirmed by immunohistochemistry (IHC) before randomization. Human epidermal growth factor receptor 2 (HER2) status was confirmed by IHC or fluorescent in situ hybridization. For biomarker analysis, IHC was undertaken using a mouse anti-human TP monoclonal antibody (Chugai Pharmaceutical Co., Japan). TP immunoreactivity was detected in the cytoplasm of carcinoma cells and semi-quantitative evaluation was undertaken using >1,000 carcinoma cells in each case. Ki67 immunostaining was performed using MIB1 monoclonal antibody (Dako Co.Ltd.) as previously described [16]. Briefly, Ki67 was stained after overnight preparation using a 1:100 dilution of the antibody. Evaluation of Ki67 was performed by counting ≥1,000 carcinoma cells from each patient in the hot spots and the percentage of immunoreactivity was subsequently determined by a labelling index [17].

Clinicopathological assessments were undertaken at the central laboratory (Department of Anatomic Pathology, Tohoku University, Graduate School of Medicine, Japan). The clinical response was evaluated in accordance with the Response Evaluation Criteria In Solid Tumors guidelines. Tumor response evaluation was performed after cycles 4 and 8, and after each cycle where possible pCR was defined as no histological evidence of invasive carcinoma, or the appearance of only non-invasive or in situ carcinoma on pathologic examination of the surgical specimen. When histological diagnosis of pCR was difficult based on hematoxylin-eosin-stained tissue sections, irrespective of whether carcinoma cells were present as ductal carcinoma in situ components, immunohistochemistry of myoepithelial markers such as cytokeratin 5/6 and p63 was used to determine the presence of invasive carcinoma [18–20]. Toxicity was graded and reported according to the NCI Common Terminology Criteria for Adverse Events version 3.

Statistical analysis

Following a reported 16 % pCR rate when FEC was followed by docetaxel alone in the JBCRG 01 trial [7], it was determined that 434 assessable patients were required for randomization to achieve 80 % power for the detection of an increase in the proportion of pCR rate of the docetaxel/capecitabine versus docetaxel group. Differences in pCR rates were calculated using a one-sided Chi square test with Schouten collection at the alpha level of 5 %; 95 % confidence interval (CI) was also calculated. In predictive factor analysis, the interaction of pCR with Ki67 as a continuous variable was explored using the subpopulation treatment effect pattern plots (STEPP) method. For each risk factor, the odds ratio (OR) for pCR and 95 % CI was calculated using simple and multivariate logistic regression models. DFS and OS were calculated using the Kaplan–Meier method. For each prognostic factor, hazard ratio (HR) for DFS and 95 % CI was calculated using the simple Cox model. Factors associated with DFS in univariate analysis were included in the multivariate Cox model.

Results

Patient population

A total of 504 patients were enrolled into the study (15 centers in Japan, 1 in China, and 1 in Hong Kong), 27 of whom withdrew during FEC therapy. Following FEC therapy, 239 patients were randomly assigned to the docetaxel/capecitabine group and 238 patients to the docetaxel alone group; all 477 patients were included in the intent-to-treat (ITT) population. Patients randomized to both groups were well balanced with respect to age, menopausal status, and baseline tumor characteristics (Table 1).

Treatment administration and study completion

No significant differences were observed in the delivery of FEC therapy between the treatment groups. However, the relative dose intensities for docetaxel were significantly lower in the docetaxel/capecitabine group than in the docetaxel alone group ($p = 0.0006$). A 25 % dose reduction was required for 33 % (79/239) of patients in the docetaxel/capecitabine group and 5.9 % (14/238) of patients in the docetaxel alone group. The rate of completion after the initial dose was significantly lower in the docetaxel/capecitabine group compared with the docetaxel alone group (44.8 and 88.7 %, respectively; $p < 0.0001$). Study discontinuation was significantly higher in the docetaxel/capecitabine (53/239; 22 %) group compared to docetaxel alone (13/238, 5.5 %; $p < 0.0001$). The majority of study withdrawals were attributed to drug toxicity (docetaxel/capecitabine: 31/53 patient; docetaxel alone: 9/13 patients; Fig. 1).

Clinical and pathological response

The overall response rate (cCR and cPR) was 88.3 % (211/239) in the docetaxel/capecitabine group and 87.4 % (208/238) in the docetaxel group; no significant differences in clinical response were noted. The proportion of BCS was 70.7 % (169/239) in the docetaxel/capecitabine group and 71.4 % (170/238) in the docetaxel group; the proportion of axillary lymph node conservation surgery was 28.9 % (69/239) and 27.7 % (66/238), respectively (data not shown).

The pCR rate was 23 % in the docetaxel/capecitabine group and 24 % in the docetaxel group ($p = 0.748$;

Table 1 Baseline patient demographics and clinical characteristics

Number	Total 504	FEC only 27	FEC + T 238	FEC + TX 239	*p* value
Age					
Median	49.0	47.0	49.0	49.0	W:0.8769
Range	25.0–70.0	28.0–65.0	25.0–68.0	25.0–70.0	
Menopausal status					
Premenopausal	282 (56.0 %)	16 (59.3 %)	133 (55.9 %)	133 (55.6 %)	C:0.9590
Postmenopausal	222 (44.0 %)	11 (40.7 %)	105 (44.1 %)	106 (44.4 %)	
Initial tumor size					
Median	3.5	3.5	3.5	3.5	W:0.7508
Range	0.8–10.5	2.0–10.5	0.8– 8.0	1.0– 9.0	
Axillary lymph nodes*					
Positive	280 (55.6 %)	12 (44.4 %)	134 (56.3 %)	134 (56.1 %)	C:0.9586
Negative	224 (44.4 %)	15 (55.6 %)	104 (43.7 %)	105 (43.9 %)	
Clinical stage					
I	5 (1.0 %)	0 (0.0 %)	2 (0.8 %)	3 (1.3 %)	C:0.9170
IIA	218 (43.3 %)	12 (44.4 %)	100 (42.0 %)	106 (44.4 %)	
IIB	226 (44.8 %)	11 (40.7 %)	110 (46.2 %)	105 (43.9 %)	
IIIA	55 (10.9 %)	4 (14.8 %)	26 (10.9 %)	25 (10.5 %)	
Histologic type					
Infiltrating ductal carcinoma	491 (97.4 %)	25 (92.6 %)	233 (97.9 %)	233 (97.5 %)	C:0.1087
Infiltrating lobular carcinoma	8 (1.6 %)	1 (3.7 %)	1 (0.4 %)	6 (2.5 %)	
Mucinous carcinoma	1 (0.2 %)	0 (0.0 %)	1 (0.4 %)	0 (0.0 %)	
Invasive micropapillary carcinoma	1 (0.2 %)	0 (0.0 %)	1 (0.4 %)	0 (0.0 %)	
Infiltrated apocrine carcinoma	2 (0.4 %)	0 (0.0 %)	2 (0.8 %)	0 (0.0 %)	
Invasive small cell carcinoma	1 (0.2 %)	1 (3.7 %)	0 (0.0 %)	0 (0.0 %)	
Histologic type					
Infiltrating ductal carcinoma	491 (97.4 %)	25 (92.6 %)	233 (97.9 %)	233 (97.5 %)	C:0.7657
Otherwise	13 (2.6 %)	2 (7.4 %)	5 (2.1 %)	6 (2.5 %)	
Nuclear grade					
G1	86 (17.1 %)	8 (29.6 %)	42 (17.6 %)	36 (15.1 %)	C:0.6716
G2	243 (48.2 %)	14 (51.9 %)	110 (46.2 %)	119 (49.8 %)	
G3	167 (33.1 %)	5 (18.5 %)	81 (34.0 %)	81 (33.9 %)	
NA/ND	8 (1.6 %)	0 (0.0 %)	5 (2.1 %)	3 (1.3 %)	
ER					
Positive	327 (64.9 %)	15 (55.6 %)	157 (66.0 %)	155 (64.9 %)	C:0.7423
Negative	163 (32.3 %)	9 (33.3 %)	75 (31.5 %)	79 (33.1 %)	
NA/ND	14 (2.8 %)	3 (11.1 %)	6 (2.5 %)	5 (2.1 %)	
PgR					
Positive	242 (48.0 %)	10 (37.0 %)	113 (47.5 %)	119 (49.8 %)	C:0.5775
Negative	246 (48.8 %)	14 (51.9 %)	119 (50.0 %)	113 (47.3 %)	
NA/ND	10 (2.0 %)	3 (11.1 %)	6 (2.5 %)	1 (0.4 %)	
ER/PgR*					
Positive	331 (65.7 %)	15 (55.6 %)	158 (66.4 %)	158 (66.1 %)	C:0.8930
Negative	159 (31.5 %)	9 (33.3 %)	74 (31.1 %)	76 (31.8 %)	
NA/ND	14 (2.8 %)	3 (11.1 %)	6 (2.5 %)	5 (2.1 %)	
HER2*					
Positive	99 (19.6 %)	7 (25.9 %)	44 (18.5 %)	48 (20.1 %)	C:0.6576
Negative	380 (75.4 %)	17 (63.0 %)	183 (76.9 %)	180 (75.3 %)	
NA/ND	25 (5.0 %)	3 (11.1 %)	11 (4.6 %)	11 (4.6 %)	

ER estrogen receptor, *FEC* fluorouracil/epirubicin/cyclophosphamide, *HER2* Human epidermal growth factor receptor 2, *NA* not available, *ND* no data, *PgR* progesterone receptor, *T* docetaxel alone, *TX* docetaxle plus capecitabine