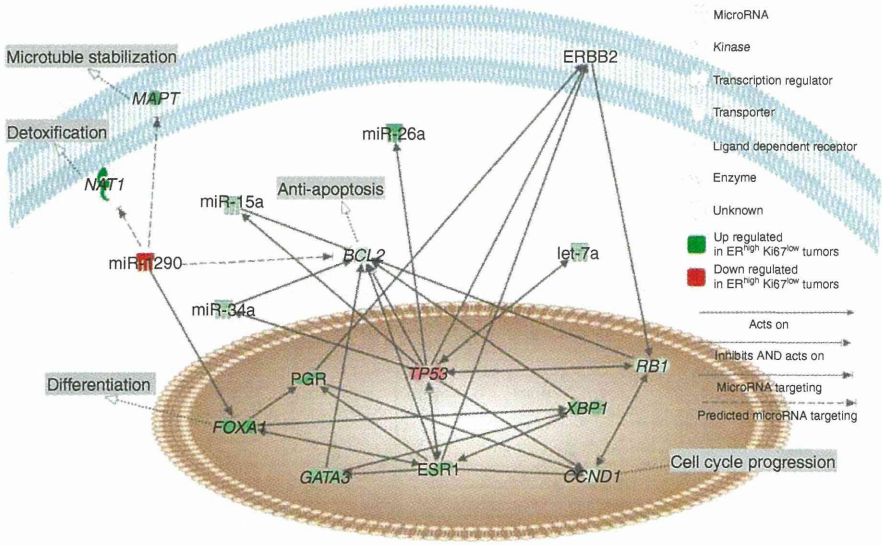

Figure 3

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

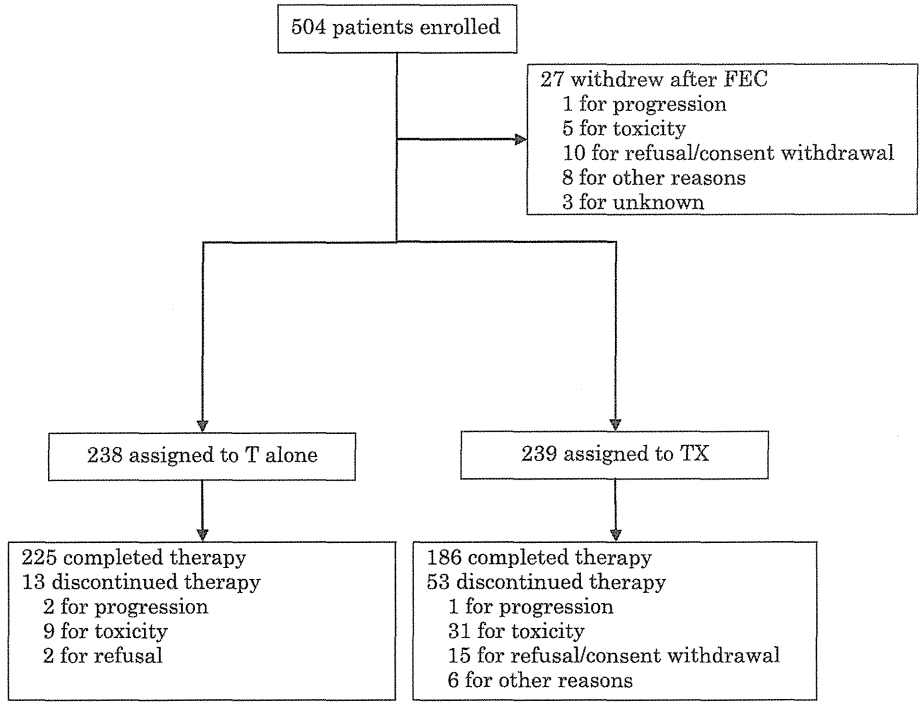

Fig. 1 Study completion. FEC: fluorouracil/epirubicin/cyclophosphamide; T: docetaxel alone; TX: docetaxel plus capecitabine

Table 2 Pathological response by (a) central assessment, (b) central assessment in patients who discontinued or received a reduced dose

	FEC (n = 27) % (95% CI)	TX (n = 239) % (95% CI)	T (n = 238) % (95% CI)	Difference (TX-T) (95 %CI)	p value
(a)					
pCR	7.4	23 (17.8–28.9)	24.4 (19.1–30.3)	−1.4 (−9.0 to 6.3)	0.7476
pINV	48.1 (28.7–68.1)	72.4 (66.3–78.0)	71.4 (65.2–77.1)	1	
Missing*	44.4 (25.5–64.7)	4.6 (2.3–8.1)	4.2 (2.0–6.7)	0.4	
(b)					
pCR	7.4	23 (17.8–28.9)	24.4 (19.1–30.3)	−1.4 (−9.0 to 6.3)	0.7476
With discontinuation		(n = 12/53)	(n = 1/13)		
pCR	–	22.6 (12.3–36.2)	7.7 (0.2–36.0)	14.9 (−3.4 to 33.3)	
With dose reduction		(n = 19/79)	(n = 2/14)		
pCR	–	24.1 (15.1–35.0)	14.3 (1.8–42.8)	9.8 (−10.8 to 30.4)	

pCR pathological complete response, *pINV* pathological presence of invasive tumor, * patients missing post-baseline mainly due to discontinuation as a result of toxicity, *CI* confidence interval, *FEC* 5-fluorouracil–epirubicin–cyclophosphamide, *TX* docetaxel plus capecitabine, *T* docetaxel alone

Table 2a). However, we observed an interesting trend in the subset of patients who had discontinued treatment or received a 25 % dose reduction. Despite treatment withdrawal, 12/53 in the docetaxel/capecitabine group and 1/13 in the docetaxel group achieved a pCR with rates of 22.6 and 7.7 %, respectively. A similar trend was observed in the 33.1 % (79/239) and 5.9 % (14/238) who received a 25 % dose reduction and achieved pCR rates of 24.1 % (19/79) and 14.3 % (2/14), respectively (Table 2b). Although not statistically significant, pCR rates were higher in the docetaxel/capecitabine group in comparison to the docetaxel group in this subpopulation.

Disease-free and overall survival

After a median 4.5-year follow-up, the 3-year DFS was estimated at 92.7 % in the docetaxel/capecitabine group and 90.7 % in the docetaxel group. Four patients were

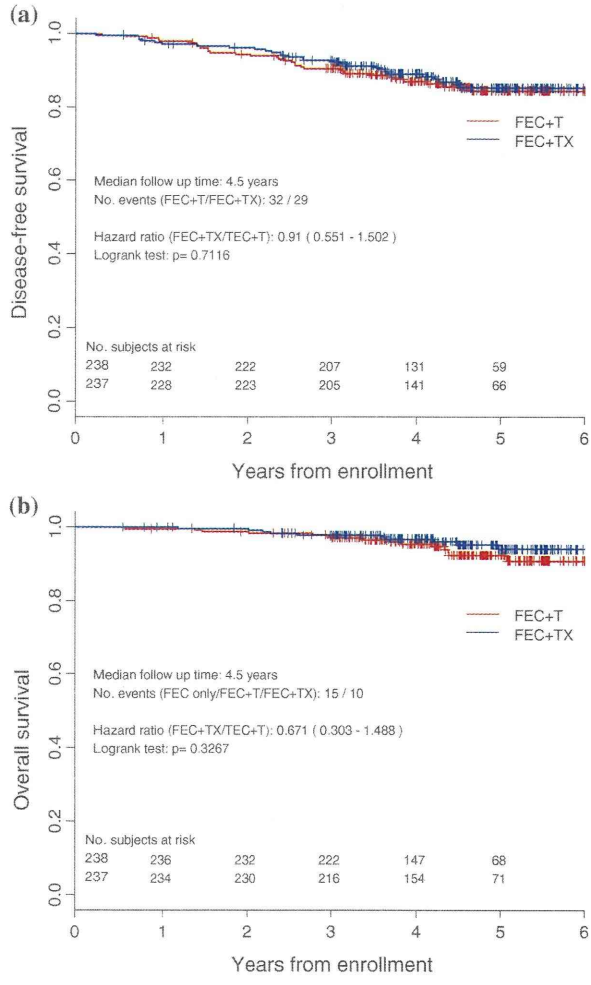

Fig. 2 **a** Disease-free survival. **b** Overall survival. FEC: fluorouracil/epirubicin/cyclophosphamide; T: docetaxel alone; TX: docetaxel plus capecitabine

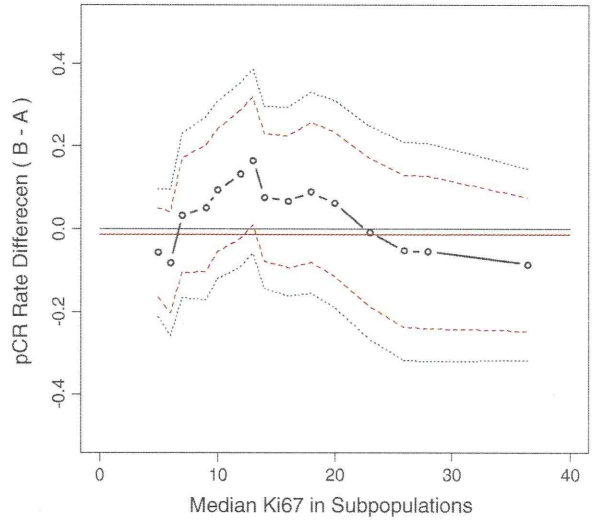

Fig. 3 STEPP analysis of the treatment effect of docetaxel/capecitabine compared with single-agent docetaxel as measured by pCR. Values >0 suggested that the combination regimen was better; <0 indicated that single-agent docetaxel was better. Difference in pCR is shown (*dashed black lines*) with corresponding 95 % CI (*dashed red lines*) and corresponding 95 % confidence band (*dashed blue lines*). Overall difference in pCR (*solid horizontal red line*) is shown

excluded from the ITT population due to missing data. A total of 29 events occurred in the docetaxel/capecitabine group and 32 in the docetaxel group, with a HR of 0.910 (95 % CI 0.551–1.502; Fig. 2a). During follow-up, 10 deaths occurred in the docetaxel/capecitabine group and 15 in the docetaxel group, with a point of estimate HR of 0.671 (95 % CI 0.303–1.488; Fig. 2b).

Predictive factor analyses for pathological response and survival status

Subpopulation analysis for pathological response showed no significant difference between treatment groups (data not shown). To identify predictive factors for pathological response using age and Ki67 as continuous variables, an overlapping subpopulation of 84 patients was constructed and analyzed using the STEPP method. Although no statistical significance was achieved, STEPP analysis indicated a trend in favor of improved pCR rate in patients with mid-range of Ki67LI (10–20 %) following docetaxel/capecitabine compared with docetaxel alone (Fig. 3). To further investigate the predictive value of Ki67 relative to pCR, univariate and multiple logistic regression models were fitted to calculate the odds ratio (OR) and 95 % CI for each risk factor.

Univariate analysis showed that nuclear grading, ER and/or PgR status, HER2 status, baseline Ki67 and TP-SI were all strongly associated with pCR (Table 3a). Multivariate analysis was performed using the predictive variables identified in the univariate analysis. To evaluate the effect of Ki67, a multivariate logistic regression analysis was undertaken in 410 patients with available baseline data for nuclear grading, ER and/or PgR, HER2, and Ki67. In the first model, all of these factors continued to be 15 % significant predictors for pCR. In the final model, pretreatment levels of Ki67 proved to be a predictive factor for pCR, with an OR of 1.031 (95 % CI 1.014–1.048; $p = 0.0004$). Using this model, the random cross-validated sensitivity and specificity were 83.3 and 63.4 %, respectively (Table 3b).

Predictive factors for DFS were analyzed using a multiple Cox model in a landmark analysis (Online Resource). When pCR and postKi67 were included in the final model, tumor stage (I, IIa/III: HR 0.144, 95 % CI 0.051–0.404; IIb/III: HR 0,264, 95 % CI 0.107–0.651; $p = 0.0006$), cancer cell TP status (continuous variables: HR 0.966,