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Original article

Anthracycline could be essential for triple-negative breast cancer: A randomised phase II study by the Kanagawa Breast Oncology Group (KBOG) 1101

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ABSTRACT

Background: It is important to determine whether anthracycline-containing regimens or taxane-containing regimens are more effective in individual patients. The present study compared the efficacy of six cycles of docetaxel and cyclophosphamide (TC6) with that of three cycles of 5-fluorouracil, epirubicin and cyclophosphamide followed by docetaxel (FEC-D) in Japanese patients with hormone receptor (HR)-negative breast cancer (BC) to identify subtypes requiring anthracycline treatment.
Methods: The study included 103 patients with operable HR-negative BC. Of these patients 53 received FEC-D and 50 received TC6. The primary endpoint was pathological complete response (pCR). The secondary endpoints were safety, breast-conserving surgery, disease-free survival (DFS) and overall survival (OS). The predictive factors for each regimen were evaluated.
Results: Of the 103 patients, 97 completed the study (FEC-D, 50 patients; TC6, 47 patients). The pCR rate was higher with FEC-D (36%) than with TC6 (25.5%); however, the difference was not significant ($P = 0.265$). TC6 was safer than FEC-D, as the adverse events with docetaxel in the FEC-D regimen were similar to those with the TC6 regimen. Among patients with basal BC, the pCR rate was significantly higher with FEC-D (42.9%) than with TC6 (13.6%; $P = 0.033$). Among patients with triple-negative breast

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cancer (TNBC), the DFS and OS were significantly better with FEC-D than with TC6 ($P = 0.016$ and $P = 0.034$, respectively).
Conclusion: TC6 was not as effective as FEC-D for treating HR-negative BC, as TC6 was not sufficient to treat TNBC, particularly the basal subtype. Our findings suggest that anthracyclines are better treatment options than taxanes for basal BC.

1. Introduction

The importance of anthracyclines in adjuvant chemotherapy for early stage breast cancer (BC) is controversial. Disease-free survival (DFS) and overall survival (OS) are reportedly longer with four-cycle regimens of docetaxel and cyclophosphamide (TC) than four-cycle regimens of anthracycline and cyclophosphamide [1]. Since the publication of these findings, the use of non-anthracycline taxane-containing regimens has become more common. However, an interim analysis of the ABC trial failed to prove the non-inferiority of non-anthracycline regimens.

Anthracycline is reportedly effective for HER2-positive BC in relation to *TOP2A* [2,3]; however, pertuzumab is presently administered in addition to trastuzumab as adjuvant therapy [4].

In contrast, cytotoxic anti-cancer drugs are currently the most accessible agents for the treatment of triple-negative breast cancer (TNBC). Recent gene expression analyses revealed that TNBC is heterogeneous and overlaps considerably with the basal-like subtype as both are characterised by the potential loss of BRCA1 function [5–7]. Further sub-classification of TNBC is an important clinical topic. Korching et al. found that cytokeratin (CK) 5/6-positive BC is negative for hormone receptors (HRs), highly proliferative and associated with specific protein expression patterns for p53 and epithelial growth factor receptor (EGFR) [8]. EGFR and CK5/6 are plausible markers for basal-like BC [9,10].

The efficacy of six cycles of TC (TC6) with that of three cycles of 5-fluorouracil, epirubicin and cyclophosphamide followed by docetaxel (FEC-D) as neoadjuvant chemotherapy (NAC) was compared in Japanese patients with HR-negative BC to identify subtypes requiring anthracycline treatment. The study further assessed the safety of TC6. To our knowledge, the efficacy and safety of TC6 in an Asian population have not been reported previously.

2. Materials and methods

2.1. Study design

This randomised, prospective, phase II study was conducted by the Kanagawa Breast Oncology Group (KBOG), which was established to perform clinical trials and improve the treatment of breast cancer in the Kanagawa prefecture, Japan. The study compared TC6 (75 mg/m^2 docetaxel and 600 mg/m^2 cyclophosphamide; TC6) with three cycles of FEC (500 mg/m^2 5-fluorouracil, 100 mg/m^2 epirubicin and 500 mg/m^2 cyclophosphamide) followed by three cycles of 100 mg/m^2 docetaxel administered intravenously on day 1 of each cycle (FEC-D). Patients were randomly assigned to receive TC6 or FEC-D. Randomisation was performed according to the HER2 status, age (<49 or ≥50 years) and hospital and was centralised at the Clinical Research Coordinating Center of Yokohama City University Medical Center. The study protocol (UMIN000002215) was approved by the institutional review board of Yokohama City University Medical Center on January 22, 2009 and subsequently by all participating institutions. Written informed consent was obtained from all participants prior to study inclusion.

2.2. Patient eligibility

Women with stage II/III BC, including those with T1cN0M0 BC, were included between February 2009 and March 2011. All patients had an Eastern Cooperative Oncology Group performance status of 0 and were 18–75 years old. Additionally, all patients were diagnosed with invasive BC via core-needle biopsy before treatment. Patients were considered ineligible if they were pregnant or nursing, and patients with T1a/T1b/N0 tumours were excluded.

2.3. Treatment

Patients received either TC6 or FEC-D every 3 weeks. Treatment was discontinued if progressive disease or unacceptable toxicity was noted. Each patient was administered 20 mg of intravenous dexamethasone 30 min before chemotherapy and 4 mg of oral dexamethasone twice daily for a total of four doses starting 1 day after each infusion. Prophylactic antiemetics were administered according to the NCCN Clinical Practice Guidelines (ver. 4, 2009) to counteract emetogenic effects. Chemotherapy was administered intravenously on day 1 of each cycle. Dose reductions were permitted, and prophylactic granulocyte colony-stimulating factor administration was allowed. Oral prophylactic antibiotics as well as postoperative radiation and treatments were used at the discretion of the treating physician. Patients were withdrawn from the study if they had grade 4 non-haematologic toxicity, progression while undergoing chemotherapy or treatment delay of >3 weeks owing to toxicity. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3).

After the operation, adjuvant therapy was administered according to attending physician judgement or patient preference.

2.4. Histopathological and immunohistological studies of pre-treatment specimens

Core-needle biopsy specimens from patients were fixed in 10% (w/v) phosphate-buffered formalin and embedded in paraffin. Sections of each block were stained with haematoxylin and eosin (H&E) and prepared for histological diagnosis. Immunohistochemical (IHC) analysis was performed as previously described [11].

The oestrogen receptor and progesterone receptor statuses were evaluated according to the Allred scoring system [12], using the ID5 and PgR636 antibodies (Dako Japan, Tokyo, Japan), respectively. IHC staining of oestrogen and progesterone receptors was considered negative when <1% of tumour cells showed positive nuclear staining. The HER2 status was examined using the Dako HercepTest™ (K5204, Dako Japan) or PathVysion™ (Abbott, Tokyo, Japan). HER2 positivity was assessed according to the American Society of Clinical Oncology/College of American Pathologists guidelines [13]. IHC staining for Ki-67 (MIB-1, Dako Japan), topoisomerase IIα (topo IIα; Ki-S1, Dako Japan) and p53 (DO-7, Dako Japan) was evaluated according to the percentage of tumour cells with positive nuclear

staining, and only unequivocal nuclear staining was considered positive. After determining the most evenly labelled tissue areas under low magnification, a minimum of 1000 tumour cells were counted at high magnification (× 400), and the number of labelled cells was calculated as a percentage of the total cell count. Tumours were further stratified into Ki-67, topo IIα and p53 high- and low-expression groups using the median expression values as cut-off points. IHC staining for CK5/6 (D5/16 B4, Dako Japan) or EGFR (3C6, Roche Diagnostics; Tokyo, Japan) was considered positive if any cytoplasmic or membranous invasive carcinoma cell staining was observed [10]. CK5/6-positive and/or EGFR-positive specimens were classified as the basal subtype. IHC staining of aldehyde dehydrogenase 1 (ALDH1; ALDHA1:EP1933Y, Abcam, Tokyo, Japan) was considered positive when ≥1% of tumour cells showed clear cytoplasmic staining [14,15].

The *TOP2A* status was examined by fluorescence in-situ hybridisation (TOP2A FISH pharmDx™ Kit; Dako Japan). The *TOP2A* copy number was determined in a minimum of 20 interphasic, non-overlapping tumour cell nuclei and was compared with chromosome 17 centromeres (*CEP17*) in those same nuclei. The ratio of *TOP2A* to *CEP17* signals (*TOP2A/CEP17*) was then calculated. A *TOP2A/CEP17* ratio ≥2.0 was considered *TOP2A* amplification [16,17].

2.5. Pathological evaluation of surgical specimens

H&E-stained slides were prepared from 5-mm tissue sections of primary tumours. Pathological breast tumour responses were assessed by two board-certified pathologists (T.S. and M.T.) blinded to treatment allocation and local assessment of response. Pathological complete response (pCR) was defined as necrosis, disappearance of all tumour cells and/or replacement of cancer cells by granulation and/or fibrosis. Pathological effects were determined using the definitions of pCR for primary tumours and lymph nodes (ypT0ypN0) [18].

2.6. Statistical methods

The primary endpoint was pCR. Sample size determinations assumed that the pCR rate of the TC6 group would be 45% [11]. Determinations also assumed that the pCR rate of the TC6 group would be superior to that of the FEC-D group by 25%. The type I error rate was set at 5% using two-sided significance tests. With a sample size of 100 patients, there was a 77% probability of rejecting the null hypothesis (no difference between the groups). The secondary endpoints were safety profiles, breast-conserving surgery rate, clinical response rate, DFS and OS. The predictive factors for each regimen were evaluated.

Patients who received at least four cycles of either regimen were included in the efficacy analysis. Those who received at least one dose of either regimen were included in the safety analysis. The pCR rates were compared between the groups using the chi-squared test. Differences, odds ratio (OR) and 95% confidence intervals (CIs) were calculated. In exploratory analysis, the pCR rates were compared between the groups for TNBC and HER2-positive cases. Breast-conserving surgery rates and clinical response rates were compared between the groups using the chi-squared test. Safety profiles were compared using Fisher's exact test. The relative dose intensity for each drug was calculated. To analyse associations between predictive factors and the pCR rate, odds ratios (ORs) and 95% CIs were calculated. This exploratory analysis was underpowered, and statistical multiplicity was not adjusted.

OS and DFS were estimated using the Kaplan–Meier method and were compared between the groups using the log-rank test when the sample size was considered sufficient.

All analyses were performed using SAS 9.3 (SAS Institute, Inc., Cary, NC). A *P*-value <0.05 was considered significant.

3. Results

3.1. Patient characteristics

The study included 103 patients from eight participating institutions. These patients were randomised to the FEC-D group (n = 53) and TC6 group (n = 50) (Table 1). Approximately 90% of the patients were histologically diagnosed with ductal carcinoma. Three patients were excluded (two with ER-positive disease and one with metastatic disease). Thus, safety was analysed in 52 patients from the FEC-D group and 48 from the TC6 group. Three patients received less than four treatment cycles and were excluded from the efficacy evaluation. Overall, 97 patients (50 patients from the FEC-D group and 47 from the TC6 group) completed the treatment and were evaluated for efficacy (Fig. 1).

3.2. Efficacy

The pCR rates were 36% and 25.5% in the FEC-D and TC6 groups, respectively (OR 1.64 95% CI 0.68–3.93; $P = 0.265$; Table 2). When TNBC was sub-classified, the pCR rate for the basal subtype was found to be significantly lower with TC6 (13.6%) than with FEC-D (42.9%; $P = 0.033$). There were no significant differences among patients with non-basal or HER2-positive disease (Table 3). The overall clinical response rate was 94.0% in the FEC-D group (CR, 42.0%; partial response [PR], 52.0%) and 93.6% in the TC6 group (CR, 38.3%; PR, 55.3%; $P = 0.938$; Table 2). No patient showed progression during treatment. The rates of breast-conserving surgery were 68.0% and 72.3% in the FEC-D and TC6 groups, respectively ($P = 0.641$; Table 2).

3.3. Safety

The mean relative dose intensities for epirubicin and docetaxel in the FEC-D regimen and docetaxel in the TC6 regimen were 96.3 ± 13.0%, 93.5 ± 14.6% and 93.9 ± 16.3%, respectively. Although no grade ≥4 adverse events were noted, grade 3 adverse events were more frequent with FEC-D than with TC6 (Table 4). Five patients (four receiving FEC-D and one receiving TC6) were hospitalised. Poor appetite, nausea, vomiting, dysgeusia, fatigue, febrile neutropenia and anaemia were more common with FEC-D than with TC6 ($P < 0.05$).

3.4. Predictive factors of pCR

Low ALDH1 expression and high topo IIα protein expression were strongly associated with pCR in the FEC-D group. ALDH1 was also associated with pCR in the TC6 group. Other factors were not associated with pCR (Fig. 2 and Fig. 3.).

3.5. Prognosis

The DFS and OS were better in patients who achieved pCR than in those who did not, although the difference was not significant ($P = 0.287$ and $P = 0.069$, respectively; Fig. 4). Of the 18 patients who developed recurrence, three patients achieved pCR and 15 patients did not. Of the three patients who achieved pCR, two with TNBC developed brain metastases and one with HER2-positive BC developed ipsilateral breast recurrence.

The DFS and OS were better with FEC-D than with TC6, although the difference was not significant ($P = 0.107$ and $P = 0.159$, respectively; Fig. 5). Among TNBC patients, the DFS and OS were

Table 1
Baseline characteristics.

		FEC-D (*N*=53)	TC6 (*N*=50)
Age (years)	Mean	54.1	53.6
	SD	12.4	10.4
ECOG PS	0	52 (98.1%)	50 (100.0%)
	1	1 (1.9%)	0 (0.0%)
Menopausal status	Pre	21 (39.6%)	17 (34.0%)
	Post	32 (60.4%)	33 (66.0%)
Clinical stage	I	5 (9.4%)	3 (6.3%)
	IIa	18 (34.0%)	16 (32.0%)
	IIb	13 (24.5%)	16 (32.0%)
	IIIa	11 (20.8%)	10 (20.0%)
	IIIb	2 (3.8%)	1 (2.0%)
	IIIc	4 (7.5%)	3 (6.0%)
	IV	0 (0.0%)	1 (2.0%)
Histological type	Ductal	48 (90.6%)	45 (90.0%)
	Lobular	2 (3.8%)	1 (2.0%)
	Other	3 (5.7%)	4 (8.0%)
Histological grade	1	7 (13.2%)	10 (20.0%)
	2	11 (20.8%)	14 (28.0%)
	3	34 (64.2%)	26 (52.0%)
Hormone receptor[a]	−	52 (98.1%)	49 (98.0%)
	+	1 (1.9%)	1 (2.0%)
HER2 status	−	38 (71.7%)	36 (72.0%)
	+	15 (28.3%)	14 (28.0%)

FEC-D: 5-fluorouracil, epirubicin and cyclophosphamide followed by docetaxel; TC6: six cycles of docetaxel and cyclophosphamide; ECOG PS: Eastern Cooperative Oncology Group performance status.
[a] Oestrogen receptor, progesterone receptor or both.

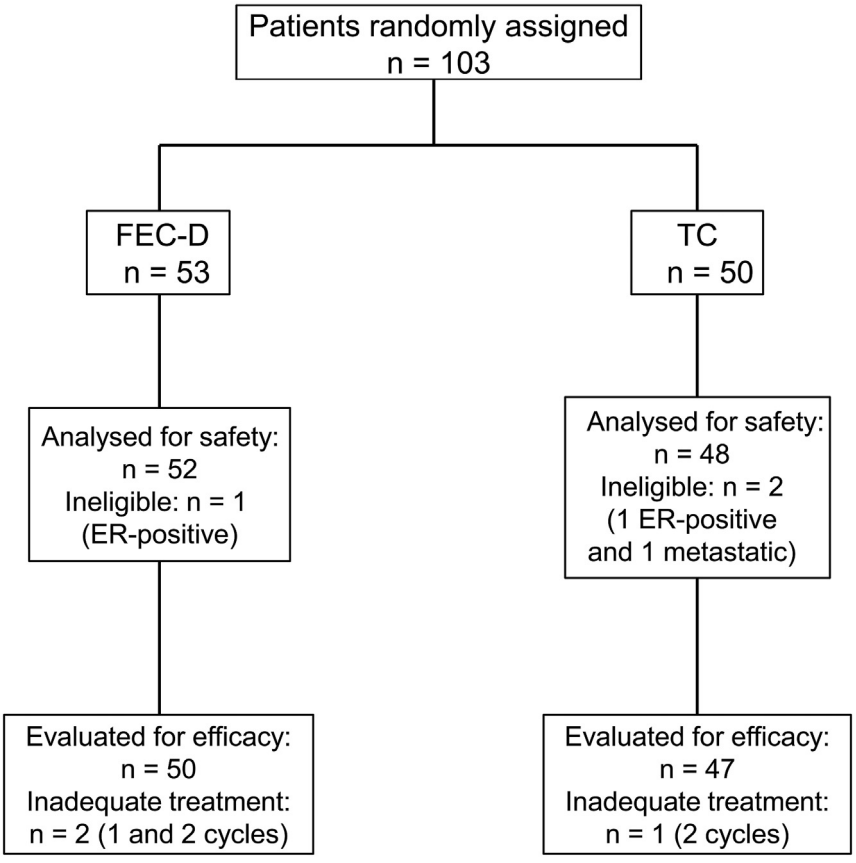

Fig. 1. CONSORT diagram.

Table 2
Clinical and pathological effects and type of surgery.

	FEC-D ($N = 50$)	TC6 ($N = 47$)	Response rate (≥PR)	
Clinical effect	*N* (%)	*N* (%)	Δ% (95% CI)	*P*-value
CR	21 (42.0%)	18 (38.3%)	⁻0.4% (⁻10.0–9.2)	0.938
PR	26 (52.0%)	26 (55.3%)		
SD	3 (6.0%)	3 (6.4%)		
PD	0 (0.0%)	0 (0.0%)		
NE	0 (0.0%)	0 (0.0%)		
Pathological effect				
pCR (ypT0ypN0)	18 (36.0%)	12 (25.5%)	10.5% (⁻7.8–28.7)	0.265
OR (95% CI)	1.64 (0.68–3.93)	1.0		
Type of surgery				
BCS	34 (68.0%)	34 (72.3%)	⁻4.3% (⁻22.5–13.9)	0.641
Mastectomy	16 (32.0%)	13 (27.7%)		

FEC-D: 5-fluorouracil, epirubicin and cyclophosphamide followed by docetaxel; TC6: six cycles of docetaxel and cyclophosphamide; CI: confidence interval; CR: complete response; NE: no effect; pCR: pathological complete response; PD: progressive disease; PR: partial response; SD: stable disease; BCS: breast-conserving surgery.

Table 3
Pathological complete response rates by subtype.

	FEC-D		TC6		*P*-value
Subtype	*N*	Grade 3	*N*	Grade 3	
Basal	21	9 (42.9%)	22	3 (13.6%)	**0.033**
Non-basal	12	3 (25.0%)	11	4 (36.4%)	0.554
HER2+	14	5 (35.7%)	12	5 (41.7%)	0.756

Significant associations are marked in boldface.
FEC-D: 5-fluorouracil, epirubicin and cyclophosphamide followed by docetaxel; TC6: six cycles of docetaxel and cyclophosphamide.

significantly better with FEC-D than with TC6 ($P = 0.016$ and $P = 0.034$, respectively; Fig. 6).

4. Discussion

In the present study, TC6 was not more efficacious than FEC-D for HR-negative BC. This finding is supported by the result of the DBCG 07-READ trial [19]. Herbeck et al. reported similar 5-year DFS and OS between TC6 and four cycles of EC followed by four cycles of docetaxel in the WSG PlanB trial [20]. An interim analysis of the ABC trial failed to prove the non-inferiority of non-anthracycline regimens. Stratified analysis of this trial showed the usefulness of anthracycline in TNBC and cases with lymph node metastasis (LNM), although the effectiveness was not demonstrated in cases with HR positivity or LNM [21]. In the ABC trial, 31% of patients had TN cancer and 60% had LNM. The high numbers of TN and LNM patients in the ABC trial might be associated with its results.

The apparent superiority of FEC-D for the basal subtype intrigues us. There are more *BRCA1*-defective tumours in the basal subtype than in the non-basal and HER2-positive subtypes [5–7]. In vitro studies have shown that resistance to single-agent taxanes is greater in *BRCA*-defective cell lines than in *BRCA* wild-type cell lines [22–24]. This finding has been clinically observed in adjuvant and metastatic settings [25,26]. *BRCA1* mutation carriers often had

Table 4
Adverse events.

	FEC-D		TC6		*P* (Grade ≥2)
	Grade 2	Grade 3	Grade 2	Grade 3	
	N (%)	*N* (%)	*N* (%)	*N* (%)	
Non-haematologic					
Poor appetite	12 (23.1%)	5 (9.6%)	2 (4.2%)	0 (0.0%)	**<0.001**
Nausea	19 (36.5%)	2 (3.8%)	0 (0.0%)	0 (0.0%)	**<0.001**
Vomiting	10 (19.2%)	2 (3.8%)	0 (0.0%)	0 (0.0%)	**<0.001**
Stomatitis	10 (19.2%)	1 (1.9%)	7 (14.6%)	0 (0.0%)	0.444
Dysgeusia	6 (11.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	**0.027**
Diarrhoea	6 (11.5%)	0 (0.0%)	6 (12.5%)	0 (0.0%)	1
Constipation	18 (34.6%)	0 (0.0%)	18 (37.5%)	0 (0.0%)	0.836
Alopecia	50 (96.2%)	0 (0.0%)	46 (95.8%)	0 (0.0%)	1
Nerve disorder	17 (32.7%)	2 (3.8%)	16 (33.3%)	3 (6.3%)	0.838
Fatigue	29 (55.8%)	1 (1.9%)	17 (35.4%)	1 (2.1%)	**0.048**
Oedema	5 (9.6%)	2 (3.8%)	9 (18.8%)	1 (2.1%)	0.426
Nail disorder	0 (0.0%)	0 (0.0%)	1 (2.1%)	0 (0.0%)	0.480
Rash	15 (28.8%)	2 (3.8%)	21 (43.8%)	2 (4.2%)	0.154
Pigmentation	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1
Cystitis	2 (3.8%)	0 (0.0%)	1 (2.1%)	0 (0.0%)	1
Allergy	0 (0.0%)	2 (3.8%)	1 (2.1%)	1 (2.1%)	1
Pain	3 (5.8%)	2 (3.8%)	3 (6.3%)	1 (2.1%)	1
Febrile neutropenia	0 (0.0%)	11 (21.2%)	0 (0.0%)	2 (4.2%)	**0.016**
Fever	5 (9.6%)	0 (0.0%)	2 (4.2%)	0 (0.0%)	0.439
Haematologic					
Leukopenia	3 (5.8%)	45 (86.5%)	5 (10.4%)	42 (87.5%)	0.364
Neutropenia	2 (3.8%)	46 (88.5%)	0 (0.0%)	47 (97.9%)	0.364
Thrombocytopenia	2 (3.8%)	0 (0.0%)	1 (2.1%)	0 (0.0%)	1
Anaemia	9 (17.3%)	0 (0.0%)	1 (2.1%)	0 (0.0%)	**0.017**

Significant associations are marked in boldface.
FEC-D: 5-fluorouracil, epirubicin and cyclophosphamide followed by docetaxel; TC6: six cycles of docetaxel and cyclophosphamide

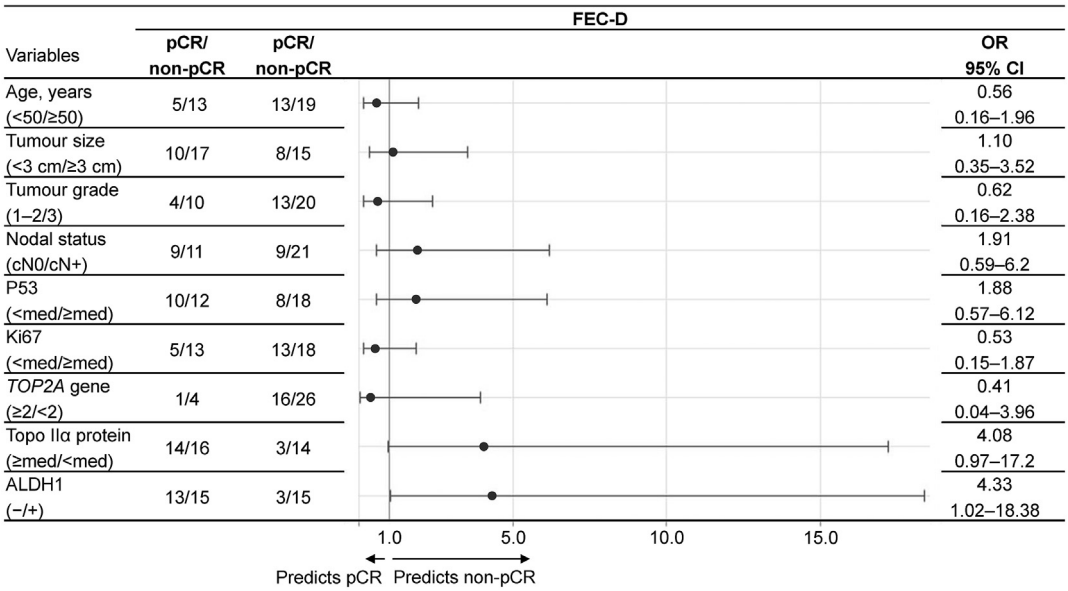

Fig. 2. Predictors of pathological complete response in patients treated with FEC-D.

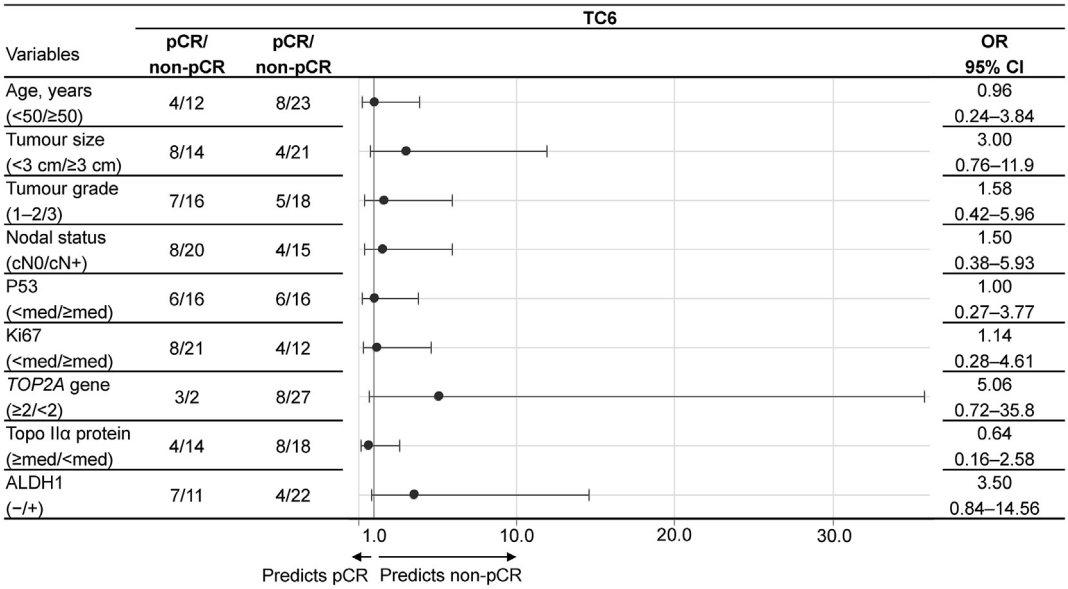

Fig. 3. Predictors of pathological complete response in patients treated with TC6.

cancer that was less sensitive to docetaxel [27]. The present study showed that TC6 was inferior for basal TNBC, which appears consistent with the association of lost BRCA1 function with decreased taxane sensitivity. Although this study was underpowered by optimistic assumptions, the obtained data were intriguing and worth sharing.

According to earlier studies in adjuvant and metastatic settings [16,17], FEC-D might be more beneficial than TC6 for HER2-positive cancers and *TOP2A* amplification might be associated with a high pCR rate in this subtype. In the present study, *TOP2A* amplification was noted in 10 of 26 HER2-positive cases (38.4%), which is consistent with previous findings [16,28–30]. However, *TOP2A* amplification was not associated with pCR, and no significant prognostic differences were observed on treatment with FEC-D. Topo IIα overexpression was associated with pCR. *TOP2A* amplification was noted in only 10 cases in this study. Other NAC studies mentioned that topo IIα overexpression is associated with anthracycline responsiveness, although these findings are contradictory [31,32]. However, identifying an optimal clinical cut-off for this protein is difficult [33].

The present study showed that TC6 was safer than FEC-D. Docetaxel in the FEC-D regimen elicited taxane-related adverse events to the same degree as it did in the TC6 regimen. Overall grade 3 adverse events were more common with FEC-D. Taken together, TC6 might be a good counterpart regimen to trastuzumab.

Tumours expressing ALDH1 exhibit stem cell properties [14,15]. In the present study, 41.9% of tumours showed ALDH1 positivity. Reportedly, the number of ALDH1-positive cells is greater in TNBC and HER2-positive tumours than in luminal cancers [14,15,34]. An association between ALDH1 and resistance to traditional

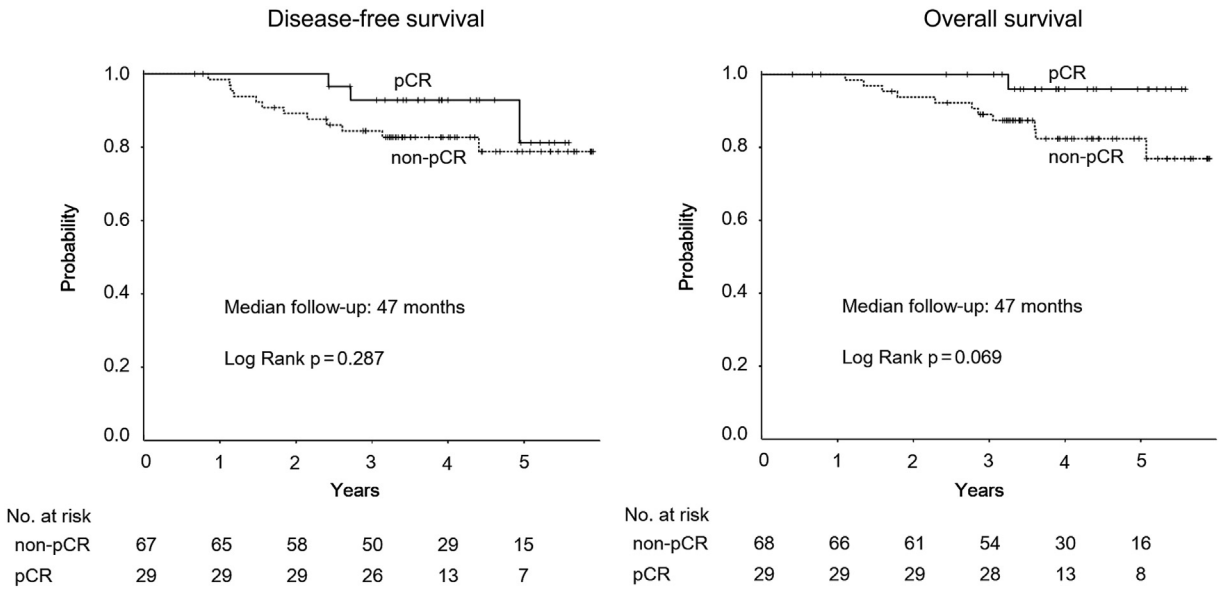

Fig. 4. Prognosis of patients who did or did not achieve pathological complete response.

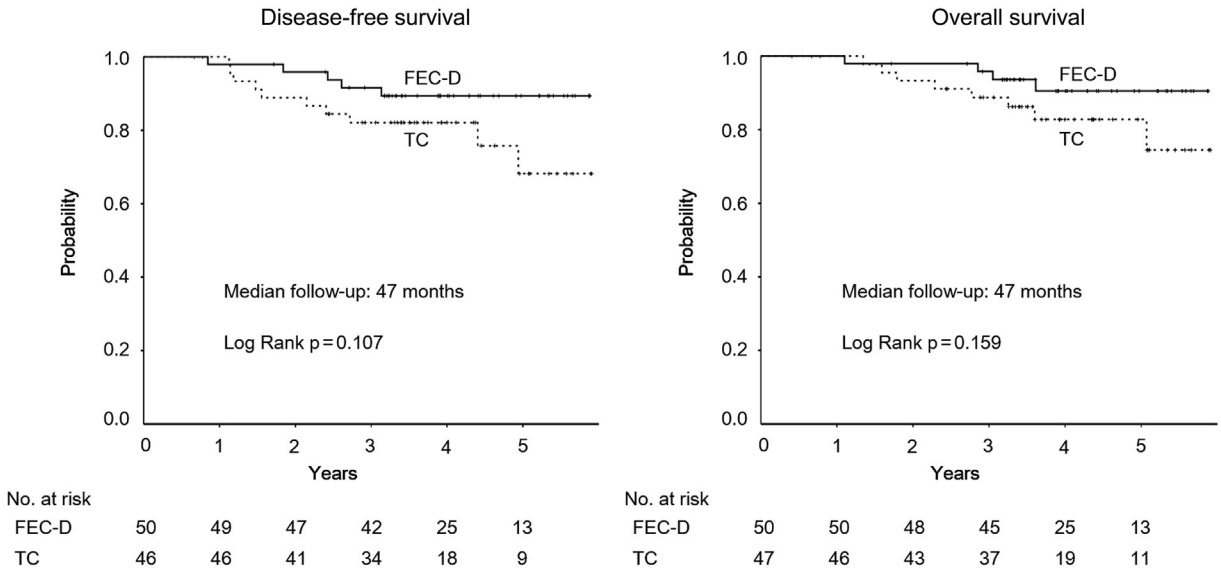

Fig. 5. Prognosis of patients according to the assigned treatment.

chemotherapy has been reported [35,36]. In the present study, ALDH1 positivity was inversely associated with pCR for both regimens, with a significant association for FEC-D. Thus, ALDH1 might be a useful marker to identify patients requiring anti-stem cell therapy. However, our previous retrospective study showed that ALDH1 had no significant impact on the prognosis of TNBC patients [37], which is similar to the finding in this study. ALDH1 should be further investigated as a biological marker of BC.

Prognosis was not significantly better in patients who achieved pCR. This finding is contrary to the result in a previous report [18]. This might be associated with the following limitations of this trial. First, the follow-up period was short. Second, the patient number was small. Third, additional treatment, including extra chemotherapy, could have affected non-pCR patients.

Patients treated with FEC-D tended to have better outcomes. Intriguingly, among TNBC patients, the prognosis was better with FEC-D. This implies that pCR could be a surrogate marker for favourable prognosis in this population. Although prognosis was not analysed for each TNBC subtype, the better prognosis among TNBC patients treated with FEC-D could be explained, at least in part, by the higher rate of pCR in TNBC patients, particularly those with the basal subtype.

5. Conclusions

TC6 was not as effective as FEC-D for treating HR-negative BC, as TC6 was not sufficient to treat TNBC, particularly the basal subtype. However, TC6 was safe and equally effective in patients with the

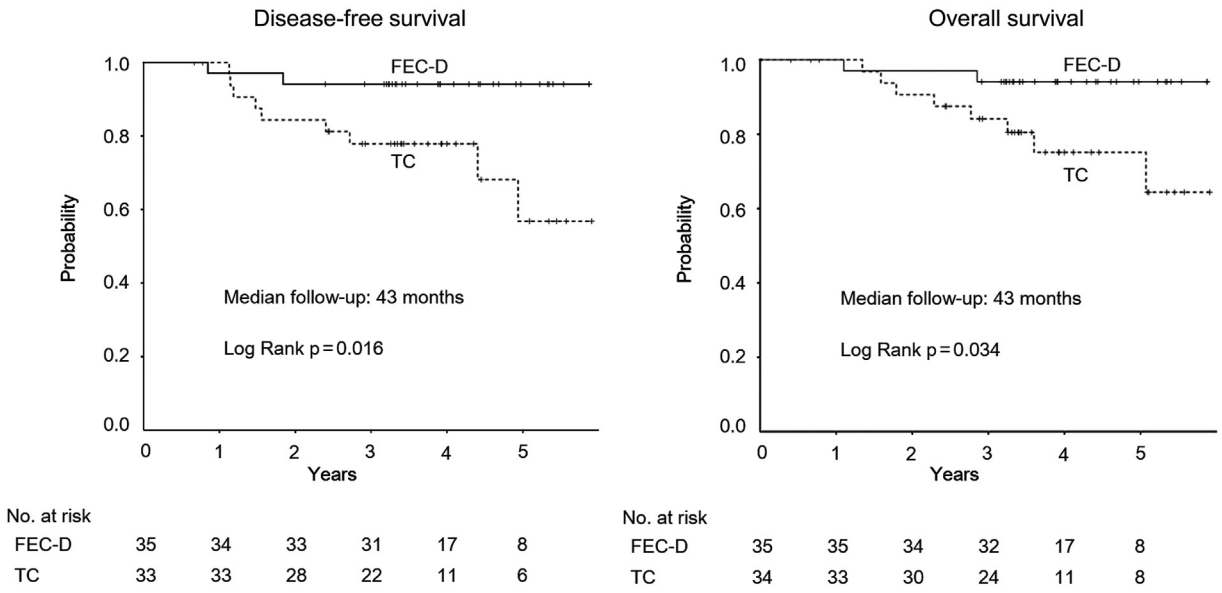

Fig. 6. Prognosis of patients with triple-negative breast cancer.

HER2-positive subtype. This study warranted confirmation in a larger randomised study of the same regimens in TNBC.

Contribution

T.I. designed this phase 2 trial. K.N., T.I., D.S., A.Y., K.K., M.M., T.D., K.T., H.O., T.O., Y.K., N.S., M.K., N.N., Y.S., Y.S., A.S., H.A., T.C., Y.I. and Y.T. were the site investigators recruited patients, contributed to patient care and collected patient data. M.T. and T.S. were the pathologists who reviewed the histopathological, immunostaining and gene expression analysis. M.S.O and S.M. were the bioinformatician who analysed and summarised the acquired data statistically. I.T. guided the initial drafting of the manuscript and K.N. drafted, refined and edited the manuscript. T.I., I.E. and Y.T. reviewed the manuscript. All authors had full access to the study data, contributed to the revision and approval of the manuscript and participated in the decision to submit the manuscript for publication.

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Conflicts of interest

The authors declare that they have no potential conflicts of interest.

Ethical standards

Experiments performed comply with current laws in Japan and written informed consent.

References

[1] Jones S, Holmes FA, O'Shaughnessy J, Blum JL, Vukelja SJ, McIntyre KJ, et al. Docetaxel with cyclophosphamide is associated with an overall survival benefit compared with doxorubicin and cyclophosphamide: 7-year follow-up of US Oncology Research Trial 9735. J Clin Oncol 2009;27:1177–83.
[2] Gennari A, Sormani MP, Pronzato P, Puntoni M, Colozza M, Pfeffer U, et al. HER2 status and efficacy of adjuvant anthracyclines in early breast cancer: A pooled analysis of randomized trials. J Natl Cancer Inst 2008;100:14–20.
[3] Pritchard KI, Messersmith H, Elavathil L, Trudeau M, O'Malley F, Dhesy-Thind B. HER-2 and topoisomerase II as predictors of response to chemotherapy. J Clin Oncol 2008;26:736–44.
[4] von Minckwitz G, Procter M, de Azambuja E, Zardavas D, Benyunes M, Viale G, et al. Adjuvant pertuzumab and trastuzumab in early HER2-positive breast cancer. N Engl J Med 2017;377:122–31.
[5] Rakha EA, Reis-Filho JS, Ellis IO. Basal-like breast cancer: A critical review. J Clin Oncol 2008;26:2568–81.
[6] Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 2003;100:8418–23.
[7] Turner NC, Reis-Filho JS. Basal-like breast cancer and the BRCA1 phenotype. Oncogene 2006;25:5846–53.
[8] Korsching E, Packeisen J, Agelopoulos K, Eisenacher M, Voss R, Isola J, et al. Cytogenetic alterations and cytokeratin expression patterns in breast cancer: Integrating a new model of breast differentiation into cytogenetic pathways of breast carcinogenesis. Lab Investig 2002;82:1525–33.
[9] Blows FM, Driver KE, Schmidt MK, Broeks A, van Leeuwen FE, Wesseling J, et al. Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: A collaborative analysis of data for 10,159 cases from 12 studies. PLoS Med 2010;7:e1000279.
[10] Cheang MC, Voduc D, Bajdik C, Leung S, McKinney S, Chia SK, et al. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. Clin Cancer Res 2008;14:1368–76.
[11] Ishikawa T, Shimizu D, Sasaki T, Morita S, Tanabe M, Ota I, et al. A human epidermal growth factor receptor 2 expression-based approach to neoadjuvant chemotherapy for operable breast cancer. Jpn J Clin Oncol 2010;40:620–6.
[12] Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 1998;11:155–68.
[13] Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 2007;25:118–45.
[14] Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 2007;1:555–67.
[15] Ricardo S, Vieira AF, Gerhard R, Leitao D, Pinto R, Cameselle-Teijeiro JF, et al. Breast cancer stem cell markers CD44, CD24 and ALDH1: Expression distribution within intrinsic molecular subtype. J Clin Pathol 2011;64:937–46.
[16] Press MF, Sauter G, Buyse M, Bernstein L, Guzman R, Santiago A, et al. Alteration of topoisomerase II-alpha gene in human breast cancer: Association with responsiveness to anthracycline-based chemotherapy. J Clin Oncol 2011;29:859–67.
[17] Slamon D, Eiermann W, Robert N, Pienkowski T, Martin M, Press M, et al. Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med 2011;365:1273–83.

[18] von Minckwitz G, Untch M, Blohmer JU, Costa SD, Eidtmann H, Fasching PA, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 2012;30:1796–804.
[19] Ejlertsen B, Tuxen MK, Jakobsen EH, Jensen MB, Knoop AS, Hojris I, et al. Adjuvant cyclophosphamide and docetaxel with or without epirubicin for early TOP2A-normal breast cancer: DBCG 07-READ, an open-label, phase III, randomized trial. J Clin Oncol 2017;35:2639–46.
[20] Harbeck N, Gluz O, Clemens MR, Malter W, Reimer T, Nuding B, et al. Prospective WSG phase III PlanB trial: Final analysis of adjuvant 4xEC -> 4x doc vs. 6x docetaxel/cyclophospham ide in patients with high clinical risk and intermediate-to-high genomic risk HER2-negative, early breast cancer. J Clin Oncol 2017;35.
[21] Blum JL, Flynn PJ, Yothers G, Asmar L, Geyer JrJr, Jacobs SA, et al. Anthracyclines in early breast cancer: The ABC trials-USOR 06-090, NSABP B-46-I/USOR 07132, and NSABP B-49 (NRG Oncology). J Clin Oncol 2017;35:2647–55.
[22] Chabalier C, Lamare C, Racca C, Privat M, Valette A, Larminat F. BRCA1 downregulation leads to premature inactivation of spindle checkpoint and confers paclitaxel resistance. Cell Cycle 2006;5:1001–7.
[23] Sung M, Giannakakou P. BRCA1 regulates microtubule dynamics and taxane-induced apoptotic cell signaling. Oncogene 2014;33:1418–28.
[24] Tassone P, Tagliaferri P, Perricelli A, Blotta S, Quaresima B, Martelli ML, et al. BRCA1 expression modulates chemosensitivity of BRCA1-defective HCC1937 human breast cancer cells. Br J Canc 2003;88:1285–91.
[25] Kriege M, Jager A, Hooning MJ, Huijskens E, Blom J, van Deurzen CH, et al. The efficacy of taxane chemotherapy for metastatic breast cancer in BRCA1 and BRCA2 mutation carriers. Cancer 2012;118:899–907.
[26] Wysocki PJ, Korski K, Lamperska K, Zaluski J, Mackiewicz A. Primary resistance to docetaxel-based chemotherapy in metastatic breast cancer patients correlates with a high frequency of BRCA1 mutations. Med Sci Monit 2008;14. SC7–10.
[27] Byrski T, Gronwald J, Huzarski T, Grzybowska E, Budryk M, Stawicka M, et al. Response to neo-adjuvant chemotherapy in women with BRCA1-positive breast cancers. Breast Canc Res Treat 2008;108:289–96.
[28] Jarvinen TA, Liu ET. HER-2/neu and topoisomerase IIalpha in breast cancer. Breast Canc Res Treat 2003;78:299–311.
[29] Jarvinen TA, Tanner M, Barlund M, Borg A, Isola J. Characterization of topoisomerase II alpha gene amplification and deletion in breast cancer. Genes Chromosomes Cancer 1999;26:142–50.
[30] Jarvinen TA, Tanner M, Rantanen V, Barlund M, Borg A, Grenman S, et al. Amplification and deletion of topoisomerase IIalpha associate with ErbB-2 amplification and affect sensitivity to topoisomerase II inhibitor doxorubicin in breast cancer. Am J Pathol 2000;156:839–47.
[31] MacGrogan G, Rudolph P, Mascarel Id I, Mauriac L, Durand M, Avril A, et al. DNA topoisomerase IIalpha expression and the response toprimary chemotherapy in breast cancer. Br J Canc 2003;89:666–71.
[32] Petit T, Wilt M, Velten M, Millon R, Rodier JF, Borel C, et al. Comparative value of tumour grade, hormonal receptors, Ki-67, HER-2 and topoisomerase II alpha status as predictive markers in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy. Eur J Cancer 2004;40:205–11.
[33] O'Malley FP, Chia S, Tu D, Shepherd LE, Levine MN, Huntsman D, et al. Topoisomerase II alpha protein and responsiveness of breast cancer to adjuvant chemotherapy with CEF compared to CMF in the NCIC CTG randomized MA.5 adjuvant trial. Breast Canc Res Treat 2011;128:401–9.
[34] Tsang JY, Huang YH, Luo MH, Ni YB, Chan SK, Lui PC, et al. Cancer stem cell markers are associated with adverse biomarker profiles and molecular subtypes of breast cancer. Breast Canc Res Treat 2012;136:407–17.
[35] Gong C, Yao H, Liu Q, Chen J, Shi J, Su F, et al. Markers of tumor-initiating cells predict chemoresistance in breast cancer. PLoS One 2010;5:e15630.
[36] Tanei T, Morimoto K, Shimazu K, Kim SJ, Tanji Y, Taguchi T, et al. Association of breast cancer stem cells identified by aldehyde dehydrogenase 1 expression with resistance to sequential Paclitaxel and epirubicin-based chemotherapy for breast cancers. Clin Cancer Res 2009;15:4234–41.
[37] Kida K, Ishikawa T, Yamada A, Shimada K, Narui K, Sugae S, et al. Effect of ALDH1 on prognosis and chemoresistance by breast cancer subtype. Breast Canc Res Treat 2016;156:261–9.