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ORIGINAL ARTICLE

Evaluation of ER and Ki-67 proliferation index as prognostic factors for survival following neoadjuvant chemotherapy with doxorubicin/docetaxel for locally advanced breast cancer

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Abstract
Background The aim of the study was to identify reliable predictive biological markers for treatment outcome following neoadjuvant adriamycin/docetaxel (AT) chemotherapy in locally advanced breast cancer patients.
Materials and methods This study was a phase II study on AT neoadjuvant chemotherapy in locally advanced breast cancer patients. Patients received 50 mg/m^2 of doxorubicin intravenously (IV) over 15 min followed by docetaxel 75 mg/m^2 infused over 1 h, repeated every 3 weeks for three cycles. Surgery was performed within 3–4 weeks following the last cycle of chemotherapy. We analyzed the pre-treatment and post-treatment expression levels of ER, PgR, HER-2, Ki-67 proliferation index, and p53 and examined the correlation between the markers and clinical parameters with treatment response, overall survival and relapse-free survival following neoadjuvant treatment.
Results From July 2001 to September 2004, 61 patients were enrolled. The meaningful parameters adversely influencing survival were post-treatment ER(−) status ($P = 0.013$) and post-treatment Ki-67 index above 1.0% ($P = 0.013$). At the multivariate level, the post-treatment Ki-67 proliferation index ≤ 1.0 was the only meaningful prognostic factor for better survival ($P = 0.033$). Notably, tumors with Ki-67 index ≤ 1.0 were more likely to express ER with statistical significance ($P = 0.002$). Tumors with ER(+) and Ki-67 index ≤ 1.0 showed the highest survival rate, followed by ER(+) and Ki-67 index > 1.0%, ER(−) and Ki-67 ≤ 1.0%, and ER(−) and Ki-67 > 1.0% with the worst survival ($P = 0.033$).
Conclusion Collectively, post-treatment ER status and Ki-67 proliferation index were prognostic of overall survival following neoadjuvant AT chemotherapy.

Keywords Ki-67 proliferation index · Breast cancer · Neoadjuvant chemotherapy

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Introduction

Neoadjuvant chemotherapy has been widely used for patient with locally advanced breast cancer over the last few decades [1]. The aims of neoadjuvant chemotherapy in locally advanced breast cancer are to reduce the size of the primary tumor, rendering breast conservation surgery possible, and to improve overall survival by eradicating micrometastatic disease [2, 3]. In addition, neoadjuvant chemotherapy offers a chance of individualization of the therapy by evaluating various biological or pathological markers for their possible predictive roles in treatment

outcome. Although a definite survival benefit has not been shown in a large trial which randomized 1,523 patients to receive an anthracycline-based chemotherapy either before surgery or in the adjuvant setting, patients who achieve a pathologic complete response (pCR) have a markedly prolonged survival than those who did not achieve pCR [4]. Similar findings have been reported in several studies [5–7].

Most trials adopted an anthracycline-based regimen for neoadjuvant chemotherapy and the reported clinical response rates (cCR) and pCR rates are in the range of 61–82% and 10–24%, respectively [4, 5, 7–12]. For metastatic breast cancer patients, anthracycline and taxanes are the two most effective antitumor drugs. There is no cross-resistance between taxanes and the anthracyclines. A pioneering dose-finding study on the combination regimen of doxorubicin and docetaxel (AT) demonstrated a cRR of 81% [13], while subsequent phase II studies showed a cRR between 70–85% [14–16].

The identification of accurate biological factors that may predict the response to neoadjuvant chemotherapy has been increasingly attracted medical oncologist's attention in the past few years. Despite of the effort directed towards the individualized pre-operative chemotherapy in breast cancer, the consensus on biological markers in this setting has not been established yet. We tested an array of biological markers that are already being widely used to select patients for adjuvant therapy, such as estrogen receptor (ER) or progesterone receptor (PgR) status [17, 18], and human epidermal growth factor-2 (HER-2) for selecting patients, who will benefit from hormonal therapy and/or trastuzumab therapy. In addition, we also evaluated pre-treatment, post-treatment expression levels and their changes of p53, and Ki-67 proliferation index for their potential role in predicting treatment response for concurrent anthracycline and docetaxel combination chemotherapy. We conducted a prospective phase II study on AT neoadjuvant chemotherapy in locally advanced breast cancer patients and investigated the pre-treatment and post-treatment expression levels of ER, PgR, HER-2, Ki-67 proliferation index, and p53 and correlated the parameters with treatment response, overall survival and relapse-free survival following neoadjuvant treatment.

Materials and methods

This was an open-label, phase II study of neoadjuvant chemotherapy with doxorubicin and docetaxel in locally advanced breast cancer patients. Sixty-one women with locally advanced breast cancer were prospectively enrolled for the study between July 2001 and September 2004. Eligible patients were required to have histologically confirmed (core or incisional biopsy) locally advanced breast cancer, no previous chemotherapy, AJCC (American Joint Committee on Cancer) clinical stage IIB-IIIC ($T \geq 5$ cm or metastasis to axillary lymph nodes), a bidimensionally measurable lesion by physical examination, ECOG (Eastern Cooperative Oncology Group) performance status of 0–2, adequate hematologic parameters (hemoglobin ≥ 9.0 g/dl, absolute neutrophil count $\geq 1{,}500\ \mu l^{-1}$; platelet count $\geq 100{,}000\ \mu l^{-1}$), renal function (creatinine clearance by Cockroft formula ≥ 50 mL/min or creatinine ≤ 1.5 mg/dl), and liver parameters (aspartate aminotransferase (AST), alanine aminotransferase (ALT) $\leq 3\times$ the upper limits of normal (ULN), total bilirubin $< 2\times$ULN). Patients with ipsilateral supraclavicular lymph nodes and/or inflammatory breast cancer were included in the study. Exclusion criteria were as follows; history of clinically significant cardiac disease as defined by symptomatic ventricular arrhythmias, congestive heart failure, or previous myocardial infarction within 12 months of study entry; active infection and psychiatric illness that would preclude obtaining informed consent; history of another malignancy within 5 years of study entry, apart from basal cell carcinoma of the skin or carcinoma in situ of the uterine cervix. All participants provided written informed consent before they entered the study in accordance with the institutional guideline.

Treatment

Patients received 50 mg/m^2 of doxorubicin intravenously (IV) over 15 min followed by docetaxel 75 mg/m^2 infused over 1 h, repeated every 3 weeks for three cycles. Surgery was performed within 3–4 weeks following the last cycle of chemotherapy. All patients with breast-conserving surgery underwent post-operative radiation therapy. Women with estrogen receptor (ER)-positive tumors received 5 years of 20 mg tamoxifen daily after surgery. Patients received adjuvant chemotherapy according to the post-operative pathologic nodal status: three cycles of AT followed by RT in case of node-negative and four cycles of AC→RT followed by four cycles of paclitaxel in case of node-positive patients. Application of docetaxel or doxorubicin was delayed as long as there was CTC grade ≥ 2 non-hematologic toxicity except for alopecia, neutropenia less than $1{,}500\ \mu l^{-1}$, or thrombocytopenia less than $100{,}000\ \mu l^{-1}$. National Cancer Institute Common Toxicity Criteria grading was recorded for each cycle.

Efficacy assessment

The primary end-point of the protocol was clinical response rate (cRR) by MRI and physical examination. The secondary end-points were pathologic complete response rate

(pCR), toxicity, and the correlation of treatment outcomes with estrogen receptor (ER), progesterone receptor (PgR), HER-2, Ki-67 growth fraction and p53. Initial evaluation included history taking, physical examination, complete blood count, chemistry, breast MRI, core or incisional biopsy, pathologic examination, chest X-ray (if suspicious of abnormal lesion, chest CT), abdominal ultrasonography and whole body bone scan. At each cycle, history taking, physical examination, complete blood count and chemistry were performed. Breast MRI was repeated following three cycles of chemotherapy to evaluate the response for neoadjuvant chemotherapy.

The clinical tumor response was assessed according to WHO criteria. The pathologic complete response (pCR) was evaluated by tumor excision and axillary lymph node dissection after three cycles of chemotherapy. Surgical specimens were reviewed by two experienced breast pathologists. In the Chevallier classification, the absence of tumor cells of the primary tumor site, or persistence of in situ disease, and negative axillary lymph nodes defined a pCR.

For the radiological tumor response, breast MRI was used for measurements of tumor. Breast MRI was performed with three-phase dynamic (one pre-contrast and two post-contrast scans after the injection of gadopentetate dimueglumine (Magnevist; BerlexLaboratories, Wayne, NJ). Images were taken in the prone position of the dedicated breast coil. MRI interpretation and measurements were performed by an experienced radiologist.

Pathological analysis and immunohistochemical studies

Pathologic diagnosis, ER, PgR, HER-2, Ki-67 growth fraction, and p53 status were assessed before the start of chemotherapy and after surgery. Tissues were routinely fixed in 10% buffered formalin and paraffin-embedded. Reagents used for immunohistochemical studies were as follows: ER (1:50 dilution, DAKO, Glostrup, Denmark), PgR (1:50, DAKO, Glostrup, Denmark), HER-2 (1:30, Zymed, San Francisco, CA, USA), Ki-67 (DAKO, Glostrup, Denmark), and p53 (1:80, Zymed, San Francisco, CA, USA). All stainings were performed on paraffin sections as previously described [19]. Briefly, paraffin-embedded tissue sections (4 μl in thickness) were placed on the silane-coated glass slides, deparaffinated in xylene, rehydrated in ethanol and washed in Tris-buffered saline. Immunostaining was performed using the avidin–biotin perioxidase complex method. Negative and positive controls were included with each assay. Tumors were considered ER- or PgR-positive if $\geq$ 10% of tumor cells were positive. A semi-quantitative scoring system (the Allred score) was used to evaluate the proportion and intensity of stained cells [20]. A total score of three or more defined positive staining. HER-2 status was scored on a scale of 0–3+ according to the Dako scoring system. The percentage of positive nuclei stained for Ki-67 was calculated each section based on the approximately 1,000 carcinoma cell nuclei. Immunoreactivity of p53 was defined as cells greater than 5% with distinct nuclear staining.

Statistical analysis

According to a Simon's two-stage phase II optimal design, a sample size of 53 was required to accept the hypothesis that the true response rate is greater than 80% with 90% power, and to reject the hypothesis that the response rate is less than 60% with 5% significance [21]. At the first stage, if there were fewer than 12 responses out of the initial 19 patients, an early termination of the study was required. Assuming that 10% of patients were not assessable, a total of 58 patients were planned to be accrued for this study. Descriptive statistics were reported as proportions and medians. Kaplan–Meier estimates were used in the analysis of time-to-event variable and the 95% confidence interval (CI) for the median time to event was computed. OS was measured from the date of diagnosis to the date of death or the last follow-up visit. Survival rates were compared for statistical differences by using log-rank analysis. Correlation analyses between the clinical and pathologic variables were performed using the two-sided *chi* square test or Fisher's exact test. Cox regression was used to delineate prognostic factors for treatment response at multivariate level.

Results

Patient characteristics

From July 2001 to September 2004, 61 patients were enrolled. The baseline characteristics are listed in Table 1. All patients had locally advanced breast cancer (AJCC stage IIB, $n = 7$ (11.5%); stage IIIA, $n = 31$ (50.8%); stage IIIB, $n = 16$ (26.2%); stage IIIC, $n = 7$ (11.5%)). The median age was 42 years (range 25–68 years). The median size of primary tumor measured by palpation was 7 cm (range 1–14 cm). Eleven (18%) patients had inflammatory cancer and seven patients (11.5%) presented with left supraclavicular lymph node enlargement. Fourteen (25.0%) patients had HER-2 3+ and five (8.9%) had HER-2 2+ tumors. The immunohistochemical stainings of Ki-67, and p53 were obtainable in 52, and 55 patients, respectively. The pre-treatment median Ki-67 index was 30 (1–95), and 30 tumors (54.5%) were p53-positive.

Efficacy and toxicity

Sixty patients, except for one, received planned three cycles of AT neoadjuvant chemotherapy. One patient received

Table 1 Patient Characteristics

	Number of patients ($n = 61$)
Median age, years (range)	42 (25–68)
Performance, ECOG	
0	29 (47.5%)
1	32 (52.5%)
Menopause status	
Premenopause	52 (85.2%)
Postmenopause	9 (14.8%)
Clinical tumor stage	
T1	3 (4.9%)
T2	9 (14.8%)
T3	34 (55.7%)
T4	15 (24.6%)
Clinical axillary LN status	
LN(−)	5 (8.2%)
LN(+)	56 (91.8%)
Clinical tumor size	
Median (range)	7 cm (1–14 cm)
Ipsilateral supraclavicular LN(+)	7 (11.5%)
Inflammatory cancer	11 (18.0%)
Tumor type	
Invasive ductal	58 (95.1%)
Invasive lobular	3 (4.9%)
Hormonal status	
ER-positive ($n = 55$)	24 (43.6%)
PgR-positive ($n = 53$)	21 (39.6%)
HER-2 status ($n = 56$)	
0–1	37 (66.1%)
2	5 (8.9%)
3	14 (25.0%)
Ki-67, median score (range) ($n = 52$)	30 (1–95)
p53 ($n = 55$)	
Positive	30 (54.5%)
Negative	25 (45.5%)

ECOG Eastern Cooperative Oncology Group, *LN* lymph node, *ER* estrogen receptor, *PgR* progesterone receptor

only one cycle due to early progression. A total of 183 cycles were administered with the median number of cycle of three per patient (1–3). Delivered relative dose intensities were 95% for both docetaxel and doxorubicin. Dose reductions were performed in four patients due to episodes of neutropenic fever. The delay of administering drugs occurred in eight patients (13.1%) mainly due to delayed recovery of cytopenia. The most common toxic effects were granulocytopenia and nausea. Grade 4 neutropenia was observed in 35 (19.1%) cycles. The incidence of neutropenic fever was 17.5% of the cycles with no toxic deaths. Grade 3 nausea and vomiting occurred in 2.7% of the cycles. No patients were discontinued from the study due to toxicities.

The overall cRR to preoperative chemotherapy was 91.8% (95%CI, 85–99) with two CRs (3.3%) and 54 PRs (88.5%). Four (6.6%) patients had stable disease and one (1.6%) patient had progressive disease during treatment. The pCR was achieved in four (7.4%) patients and the mean pathologic tumor size was 2.9 cm (range, 0.0–10.0). The overall radiologic RR was 70.0% with one CR and 35 PRs. Of the 61 patients, 57 patients underwent curative breast cancer surgery following AT: 42 (73.7%) patients received modified radical mastectomy; 15 (26.3%) breast conserving surgery; two refused surgery; one was lost to follow-up; and one had early progression of the disease during AT chemotherapy. With median follow-up duration of 37.9 months, median overall survival has not been reached yet, and median time to progression was 40.2 months (95% CI 32.4–48). At this writing, 23 patients have relapsed with 19 (83%) systemic relapses and 3 (13%) locoregional recurrences. The sites of systemic recurrence included bone ($n = 8$, 34.8%), lung ($n = 4$, 17.4%), skin ($n = 4$, 17.4%), and liver ($n = 3$, 13.0%). Fifty-seven patients received adjuvant chemotherapy with or without radiotherapy (AC→RT→paclitaxel, $n = 20$; AT→RT, $n = 30$; AT alone, $n = 4$; others, $n = 2$). Different adjuvant treatment modalities did not influence on survival or relapse-free survival (AC→RT→paclitaxel vs. AT→RT), $P = 0.789$.

Pre-treatment biological markers and treatment response

The pre-treatment biological markers such as ER, PgR, HER-2, Ki-67, or p53 were not correlated with clinical, pathological or radiological responses (Table 2).

Changes in biological markers during treatment and treatment response

Of the 48 patients with both pre- and post-treatment ER results available, nine (18.8%) patients showed increase in ER score and three (6.3%) patients demonstrated a shift from ER− to ER+ tumor (Table 3, Fig. 1). The alterations in ER score did not correlate with clinical, pathological or radiological response ($P = 0.316$, 0.247, 0.143, respectively). Although a positive conversion of the ER status after chemotherapy observed in three cases, the actual Allred scores in positively converted tissues were three in all three cases. Likewise, changes in PgR scores did not influence clinical or pathological response (Table 3, Fig. 1). Most of the tumors retained its HER-2 expression after treatment. The median Ki-67 proliferation index was dramatically decreased after neoadjuvant chemotherapy from 30% (range 1–95) to 1% (range 0–95) with the median

Table 2 Response rates by pre-treatment marker status

	cRR (%)	*P* value	pCR (%)	*P* value	Radiological RR (%)	*P* value
Hormonal status						
ER(+)	22/24 (91.7)	0.863	1/23(4.3)	0.435	14/22 (63.6)	0.557
ER(−)	28/31 (90.3)		3/28 (10.7)		18/25 (72.0)	
PgR(+)	19/21 (90.5)	0.986	1/20 (5.0)	0.534	12/19 (63.2)	0.566
PgR(−)	29/32 (90.6)		3/29 (10.3)		19/26 (73.1)	
HER-2 status						
0–2	37/41 (90.2)	0.769	4/37(10.8)	0.225	23/34 (67.6)	0.811
3	13/14 (92.9)		0/14 (0.0)		9/13 (69.2)	
Ki-67						
> 1	43/48 (89.6)	0.497	4/48 (8.3)	0.548	31/43 (72.1)	0.526
≤ 1	4/4 (100.0)		0/4 (0.0)		1/4 (25.0)	
p53						
Positive	28/30 (93.3)	0.554	3/30 (10.0)	0.353	16/23 (69.6)	0.583
Negative	24/27 (88.9)		1/27 (3.7)		16/24 (66.7)	

cRR Clinical response rate, *pCR* pathologic response rate, *ER* estrogen receptor, *PgR* progesterone receptor

Table 3 Response rates by changes in marker status

	cRR (%)	*P* value	Radiological RR (%)	*P* value
Hormonal status				
↑ER score	9/9 (100.0)	0.316	3/7 (42.9)	0.143
↓,→ ER score	35/39 (89.7)		25/36 (69.4)	
↑PgR score	8/8 (100.0)	0.337	3/7	0.159
↓,→ PgR score	34/38 (89.5)		24/34	
Her-2 status				
↑Her-2	12/13 (92.3)	0.901	7/12	0.561
↓,→ Her-2	31/34 (91.2)		21/31	
Ki-67				
↑Ki-67	10/11 (90.9)	0.978	7/10	0.572
↓,→ Ki-67	31/34 (91.2)		18/30	
p53				
↑p53	4/4 (100.0)	0.670	2/2	0.298
↓,→ p53	44/46 (95.7)		27/42	

cRR Clinical response rate, *pCR* pathologic response rate, *ER* estrogen receptor, *PgR* progesterone receptor

reduction in Ki-67 proliferation index of nine ($P = 0.004$). However, there was no correlation between reduction in Ki-67 proliferation index and clinical response or pathological response ($P = 0.978$, 0.411, respectively). Overall, changes in PgR score, HER-2 score, Ki-67 proliferation index, and percentage of p53 staining did not predict clinical or pathological response.

The effect of post-treatment ki-67 proliferation index, and ER status on survival

Various clinical and pathologic variables were tested for prognostic factors for poor survival or recurrence at univariate level using log-rank tests (Table 4). The meaningful parameters adversely influencing survival were post-treatment ER (−) status ($P = 0.017$) and post-treatment Ki-67 index above 1.0% ($P = 0.013$). Other variables such as age, menopausal status, performance status, inflammatory cancer, ipsilateral supraclavicular lymph node, tumor size, nuclear grade, pretreatment biological markers, post-treatment PgR or p53 did not significantly influence survival or relapse-free survival at univariate level (Table 4). At the multivariate level, the post-treatment Ki-67 proliferation index ≤ 1.0 was the only meaningful prognostic factor for better survival ($P = 0.033$).

The correlations among ER, Ki-67 proliferation index and tumor size are shown in Table 5. Notably, tumors with Ki-67 index ≤ 1.0 were more likely to express ER with statistical significance ($P = 0.002$). The post-treatment Ki-67 proliferation index did not significantly correlate with the initial tumor size. Survival rates differ according to the status of post-treatment ER and Ki-67 index (Fig. 2). Tumors with ER(+) and Ki-67 index ≤ 1.0 showed the highest survival rate, followed by ER(+) and Ki-67 index > 1.0%, ER(−) and Ki-67 ≤ 1.0%, and ER(−) and Ki-67 > 1.0% with the worst survival ($P = 0.033$).

Discussion

The aim of the study was to identify reliable predictive biological markers for treatment outcome following neoadjuvant AT chemotherapy. The ER-negative status has been correlated with a better response to preoperative chemotherapy in several studies [22–24]. Contrary to the results from previous studies, there was no difference in clinical response rate according to the hormonal receptor status.

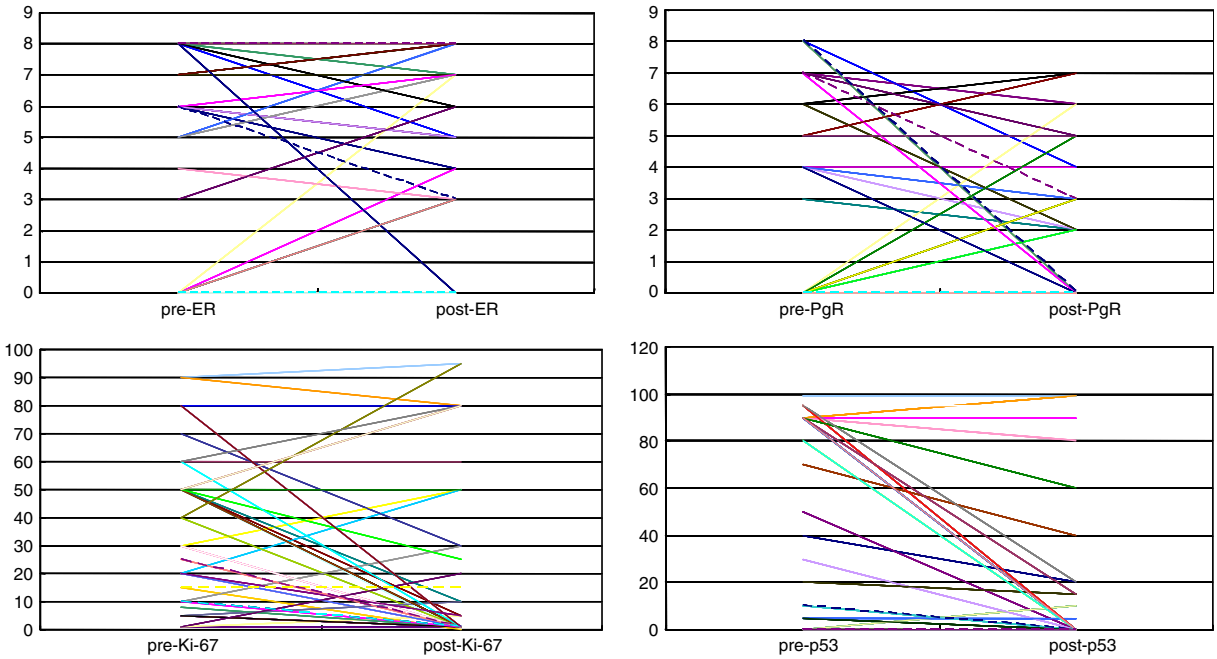

Fig. 1 Changes in marker status after neoadjuvant chemotherapy

Table 4 Univariate analyses of effects of patients and tumor variables on overall survival and relapse free survival

Parameter	Overall survival (*P* value)	Relapse free survival (*P* value)
Age > 50	0.776	0.877
Menopause	0.974	0.839
ECOG performance status	0.219	0.974
Inflammatory breast cancer	0.575	0.436
Ipsilateral supraclavicular LN	0.708	0.653
Tumor size	0.510	0.209
Nuclear grade	0.511	0.146
Adjuvant therapy (AT/RT vs AC/RT/paclitaxel)	0.789	0.912
Pre-tx ER expression	0.137	0.141
Pre-tx PgR expression	0.104	0.174
Pre-tx Her-2 expression	0.979	0.713
Pre-tx Ki-67index	0.812	0.375
Pre-tx p53 expression	0.613	0.899
Post-tx ER expression	0.017	0.072
Post-tx PgR expression	0.053	0.105
Post-tx Her-2 expression	0.133	0.503
Post-tx Ki-67index	0.013	0.107
Post-tx p53 expression	0.857	0.385

ECOG Eastern Cooperative Oncology Group, *LN* lymph node, *AT/RT* adriamycin/taxotere, *pre-tx* pre-treatment, *post-tx* post-treatment

Other markers such as PgR, HER-2, Ki-67, or p53 were not predictive of clinical or pathologic response. Ki-67 is a cell cycle marker that is frequently evaluated in neoadjuvant setting for its potential role as a surrogate endpoint. Recently, neoadjuvant letrozole treatment was markedly associated with a prominent reduction in the Ki-67 proliferation index [25–27]. The median Ki-67 proliferation index was dramatically reduced after neoadjuvant chemotherapy from 30% (range 1–95) to 1% (range 0–95) with the median reduction in Ki-67 proliferation index of nine, which suggests that chemotherapy is exerting an anti-proliferative effect on tumors. Fifty percent of the tumors showed less than 1.0% for Ki-67 index, which refers to cell cycle arrest. A recent analysis demonstrated the correlation between a cell cycle CR, defined as a post-treatment Ki-67 of $\leq 1.0\%$, and the ER expression [28]. In this study, the post-treatment Ki-67 proliferation index $\leq 1.0\%$ was the only meaningful prognostic factor for better survival ($P = 0.033$) at multivariate level.

In agreement with previous studies [28, 29], tumors with Ki-67 index ≤ 1.0 were more likely to express ER with statistical significance ($P = 0.002$). The ER status and Ki-67 index were shown to be the two important markers independently associated with tumor grade [29]. Despite the fact that the normal breast epithelium is stimulated by estrogen for growth, few groups observed complete dissociation between ER expression and Ki-67 index in the same normal breast cell [30, 31]. Most of the post-treatment ER(+) tumors were negative for the Ki-67 proliferation index. Interestingly, survival rates differed according to the status of post-treatment ER and Ki-67 index with ER(+) and Ki-67 index $\leq 1.0\%$ tumors being the most favorable and ER(−) and Ki-67 index $> 1.0\%$ pursuing the poorest

Table 5 Correlations among ER, Ki-67 and tumor size

Ki-67 index	Post-tx ER expression		Initial tumor size	
	(+)	(−)	T1/T2	T3/T4
≤ 1.0	20/28 (71.4)	7/24 (29.2)	5/9 (55.6)	22/44 (50.0)
1.1–50.0	7/28 (25.0)	7/24 (29.2)	3/9 (33.3)	12/44 (27.3)
50.1–100.0	1/28 (3.6)	10/24 (41.7)	1/9 (11.1)	10/44 (22.7)
P value	0.002		0.731	

Post-tx post-treatment

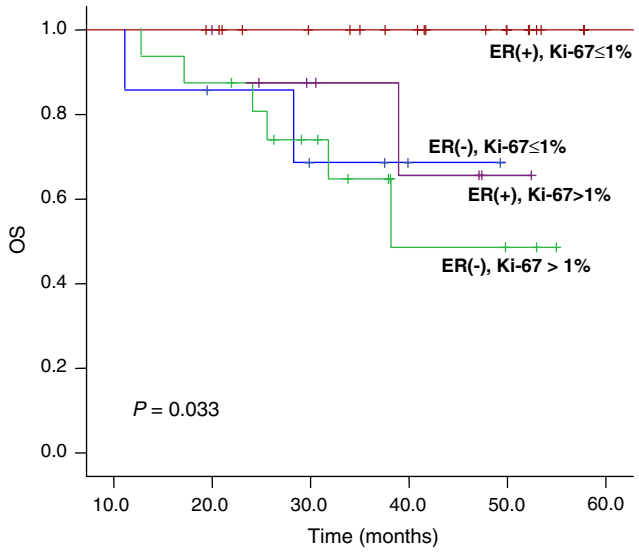

Fig. 2 Survival according to post-treatment ER and Ki-67 proliferation index

survival with statistical significance ($P = 0.033$). Thus, post-treatment assessments of Ki-67 index and ER status may have a promising role in predicting survival following neoadjuvant chemotherapy.

In terms of the efficacy, the current study demonstrated that the neoadjuvant AT in stage IIB–IIIC breast cancer patients were active with a cRR of 91.8% (95% CI, 85–99). The main toxicity was granulocytopenia, but was easily managed at the outpatient clinics. The compliance was very high with a relative dose intensity of each drug of 0.95. However, the pCR rate (7.4%) and the breast-conserving surgery rate (26.3%) in this trial were somewhat lower than those reported in previous studies [9, 16, 32–34]. One of the plausible reasons for the lower rates of pCR and breast-conserving surgery rate may be the large tumor size (median 7 cm, range 1–14 cm) in our study. Moreover, 18.0% of the enrolled patients had inflammatory cancer, while 11.5% of the patients had ipsilateral supraclavicular lymph node metastases (N3 disease) at study entry. Most of the previous trials included patients with ≤ N2 disease but not N3 [9, 16].

The optimal sequence, intensity and duration of the AT chemotherapy need to be defined in neoadjuvant setting for breast cancer. The Anglo–Celtic Cooperative Oncology Group (ACCOG) trial randomized 363 patients with primary tumors > 3 cm or inflammatory or locally advanced breast cancer to six cycles of pre-operative adrimycin/cyclophosphamide (AC) or AT. After a median follow-up of 32 months, there were no differences in cRR (61 vs. 70%), cCR rate (17 vs. 20%), the breast-conserving surgery rate (20 vs. 20%), pCR rate (24 vs. 21%), or relapse rate (31 vs. 25%) between the AC and AT groups [9]. The German Pre-operative Adriamycin and Docetaxel study II (GEPARDUO) randomized 913 patients with T2-3N0-2 breast cancer to four cycles of neoadjuvant AT every 2 weeks versus four cycles of AC every 3 weeks followed by four cycles of docetaxel (AC→D) [35]. Sequential AC→D showed a higher cRR (85 vs. 75%, $P < 0.001$), pCR (22 vs. 11%, $P < 0.001$), and the breast-conserving surgery rate (75 vs. 66%, $P < 0.005$) than dose-dense AT. Recently, a small randomized study of 45 patients to compare three versus six cycles of AT reported a higher pCR rate (10 vs. 36%, $P = 0.045$) in the six cycle arm than that in the three cycle arm, although it is inconclusive due to a small sample size [36]. The high frequency of systemic relapse (83%) following AT chemotherapy observed in the current study may prompt a need for more intense systemic therapy or longer duration of chemotherapy.

In conclusion, post-treatment ER status and Ki-67 proliferation index were the two important biomarkers that were prognostic of overall survival following neoadjuvant chemotherapy in locally advanced breast cancer patients. The chemotherapy markedly reduced the proliferation index in tumor and most post-treatment ER (+) tumors were negative for the proliferation index. The role of cell cycle CR using Ki-67 index as a surrogate endpoint in neoadjuvant AT chemotherapy should be investigated in future studies.

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Conflict of Interest Authors claim no conflicts of interest.

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