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Original Article

Neoadjuvant Chemotherapy with or without Concurrent Hormone Therapy in Estrogen Receptor-Positive Breast Cancer: NACED-Randomized Multicenter Phase II Trial

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Although in the neoadjuvant setting for estrogen receptor (ER)-positive breast cancers, chemotherapy or hormone therapy alone does not result in satisfactory tumor response, it is unknown whether concurrent chemo-endocrine therapy is superior to chemotherapy alone in clinical outcomes. We conducted a randomized phase II trial to test the responses of ER-positive patients to concurrent administration of chemo-endocrine therapy in the neoadjuvant setting. Women with stage II-III, ER-positive, invasive breast cancer (n=28) received paclitaxel followed by fluorouracil, epirubicin, cyclophosphamide (T-FEC) and were randomized to receive concurrent chemo-endocrine therapy consisting of goserelin administered subcutaneously for premenopausal women or an aromatase inhibitor for postmenopausal women. The primary endpoint was the pathological complete response (pCR) rate after neoadjuvant therapy. Twenty-eight patients were randomized. There were no significant differences in pCR rate between the concurrent group (12.5%; 2/16) and the chemotherapy alone group (8.3%; 1/12). Tumor size after therapy was significantly reduced in the concurrent therapy group (p=0.035), but not in the chemotherapy-alone group (p=0.622). Neoadjuvant chemotherapy with concurrent hormone therapy provided no significant improvement in pCR rate in ER-positive breast cancers. These preliminary results should be followed up by further studies.

Key words: breast cancer, neoadjuvant chemotherapy, concurrent hormone therapy, estrogen receptor positive, tumor response

Neoadjuvant chemotherapy (NAC) has become standard clinical practice for the treatment of breast cancer. Results from a large randomized trial showed that overall survival (OS) and disease-free survival (DFS) in patients with early breast cancer

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did not depend on whether chemotherapy was given in the neoadjuvant or adjuvant setting [1]. Higher rates of pathological complete response (pCR) have been reported in triple-receptor-negative breast cancers, human epidermal growth factor receptor 2 (HER2)-positive breast cancers, high-grade breast cancers, and breast cancers in younger women [2–6]. Higher pCR rates were also associated with breast cancers with a high recurrence score. For breast cancers that are strongly ER-positive the benefit in terms of pathological complete response is limited. [5, 7]. However, neoadjuvant chemotherapy has become standard therapy for breast cancers with high risk for recurrence. Novel strategies are needed to improve the overall tumor response in ER-positive patients and the high-recurrence-risk group, such as those with lymphatic tumor emboli (+). Traditionally, neoadjuvant endocrine therapy has been reserved for locally advanced breast cancers, often in older patients with co-morbidities. However, in recent times several clinical trials have examined this approach in patients with operable breast cancer and have demonstrated the feasibility of this low-toxicity approach in postmenopausal women [8–12].

Whether to add chemotherapy to endocrine therapy is attractive in theory [13], but there is no consensus regarding tamoxifen as well as other selective estrogen receptor modulators (SERMs) [14]. Previous clinical studies have found that concurrent tamoxifen and chemotherapy resulted in inferior outcomes compared to the sequential therapy [14–18]. Moreover, interference with drug-induced cytotoxicity has been found *in vitro* when tamoxifen is added to cancer cell lines concurrently with chemotherapy [15, 19, 20]. As a result of these studies, concurrent chemotherapy and tamoxifen therapy are avoided. Recent preclinical data by Tabuchi *et al.* have also shown that Bcl-2 overexpression through an ER-mediated pathway by E2 treatment caused resistance to paclitaxel in ER-positive breast cancers [21]. However, Ikeda *et al.* showed the synergistic effects of aromatase inhibitor (AI) with chemotherapy, in contrast to the antagonistic effects of tamoxifen with chemotherapy [22], and Sui *et al* also reported that the addition of fulvestrant could completely reverse the resistance of ER-positive cells to paclitaxel, vinblastine and vinorelbine [23, 24]. They hypothesize that a combination of a cytotoxic and an endocrine agent could have synergistic effects in ER-positive cancers.

To examine this hypothesis, we conducted this trial in the neoadjuvant setting to assess the efficacy of concurrent estrogen deprivation therapy and chemotherapy for ER-positive cases.

Materials and Methods

Patient cohorts. The study was conducted at 5 referral hospitals including Chugoku Central Hospital, Okayama University Hospital, Hiroshima City Hospital, Onomichi City Hospital, and Okayama Central Hospital. To be eligible, patients had to be ≥ 20 years of age, ECOG performance status 0 or 1, histologically confirmed invasive breast cancer with a clinical stage of T1-4, N0-3, and they had to have received no treatment for the current breast cancer. A prior history of hormone replacement therapy was allowed, provided it had been discontinued 6 months before the diagnosis of the current breast cancer. Patients were excluded if they were pregnant or breast feeding, had a history of severe allergic reactions, an allergy to the surfactant cremophor, paclitaxel (T), 5-fluorouracil (F), epirubicin (E), cyclophosphamide (C), LHRH analogues, exemestane, or alcohol. Patients were also excluded if they had multiple active malignancies.

Pretreatment core-needle-biopsy specimens and surgical specimens of the primary cancer after neoadjuvant therapy alone were collected between April 2007 and March 2009; then the period of enrollment was extended due to low numbers of patients. The study was approved by the Institutional Review Board at each institution and all patients signed an informed consent before therapy. The study was a neoadjuvant phase II randomized multicenter clinical trial (NACED: UMIN000000748, http://www.umin.ac.jp/) in ER-positive breast cancer to assess the efficacy of estrogen deprivation therapy through the concurrent administration of chemotherapy plus a luteinizing hormone-releasing hormone (LHRH) analogue in premenopausal women or an aromatase inhibitor in postmenopausal women versus chemotherapy alone.

Histopathology and scoring of biomarkers. We obtained core-needle-biopsy specimens before therapy (*i.e.*, at baseline) and excision samples at surgery. Modified Black's nuclear grades were used (grades 1 to 3). ER and Progesterone receptor (PR)

status was classified according to the Allred score or immunohistologically (IHC). For Allred scores 3–8 or in IHC [25], having > 10 percent positive tumor cells is described as positive. HER2 was scored by Hercep Test (DAKO Glostrup Denmark), using a 0–3 scale, based on the staining intensity of tumor cells. Cases that showed either staining score 3 or HER2 gene copy number > 2.0 by fluorescent *in situ* hybridization (FISH) analysis were considered HER2-positive as per ASCO guidelines [26, 27]. Immunohistochemical (IHC) staining for Ki67 was used to establish a proliferation index and was assessed pre- and post-neoadjuvant therapy. The IHC-Ki67 assay was performed using the MIB-1 antibody (DAKO Glostrup Denmark) on the Immunostainer system (DAKO Glostrup Denmark). To score Ki67, photomicrographs were taken under 40× magnification and the percentage of Ki67-positive cells was scored. Where possible, 1,000 malignant cells (at least 500 cells) in the invasive carcinoma cells area were viewed.

Residual disease (RD) after neoadjuvant therapy includes a broad range of actual responses from near pCR to no change in the size of the tumor to outright resistance to treatment. For evaluation of the response to chemotherapy with or without endocrine therapy we calculated RCB, which is a validated significant predictor of disease-free survival after neoadjuvant chemotherapy [28]. We obtained the pathological variables (the bidimensional diameters of the primary tumor bed in the resection specimen, the proportion of the primary tumor bed that contains invasive carcinoma, the number of metastatic axillary lymph nodes and the diameters of the largest metastatic axillary lymph nodes) and entered these data into the online RCB tool (http://www.nuverabio.com/other/rcb/rcbIndex.html accessed date: April, 2011). The RCB index was classified into RCB-0, I, II and III. An RCB index of 0 (=pCR) contains no invasive or *in situ* disease in the breast or axillary lymph nodes.

Treatment. All patients received 80 mg/m^2 of paclitaxel (T) given weekly for 12 weeks followed by a combination of fluorouracil (F) 500 mg/m^2, epirubicin (E) 100 mg/m^2 and cyclophosphamide (C) 500 mg/m^2 (FEC) given every 3 weeks for 4 cycles. Premenopausal women were randomized to receive chemotherapy alone or chemotherapy plus a subcutaneous injection (3.75 mg) of the LHRH analogue, LEUPLIN® (Takeda; Osaka, Japan) every 28 days for 6 months, beginning within 2 weeks of the first T treatment. Postmenopausal women were randomized to receive chemotherapy alone or chemotherapy plus 25 mg daily of a steroidal AI, exemestane, initiated within 2 weeks of the first T for 6 consecutive months until one day before operation.

Toxicity and tumor measurements were recorded during each cycle of therapy. Adverse events were recorded using Common Terminology Criteria for Adverse Event (CTCAE) version 3.0 or 4.0. Treatment was discontinued if severe toxicity was reported as probably or definitely related to treatment, or if the patients withdrew consent.

Statistical analysis. The primary objective of the study was to compare the pCR rates after neoadjuvant chemotherapy between neoadjuvant chemotherapy alone and that in combination with concurrent estrogen-deprivation treatment by either an LHRH-agonist or AI in patients with ER-positive breast cancer. The planned number of cases was 120 based on the Bayesian method with an 80% confidence level and 15% margin of error [29]. The two secondary endpoints were tumor response, determined by change in tumor size as assessed by calipers or ultrasound, and histopathological response as assessed by change in IHC-Ki67 and RCB [28]. Because clinical outcomes in ER-positive cancers are distinct by proliferative level, we assessed the efficacy by proliferative level, and divided the ER-positive cohort into high- and low-IHC-Ki67 groups (cutoff point for IHC-Ki67 level, 13.25%, as previously described by Cheang *et al.* [30]). Statistical analyses were performed with R version 2.10.0 (http://www. r-project. org/ accessed date: October, 2011). For continuous variables we used the Wilcoxon test and for categorical variables we used the Fisher exact test. Two-sided p-values < 0.05 were considered statistically significant.

Results

In the original plan, we set the target sample size as 120 cases in 2 years. The accumulated cases had not reached this number in 2 years, so we decided to extend the study for another 2 years. Although we had also set an interim analysis at the time that 60 cases were enrolled, this number of cases was never reached, so the early step was skipped. A total of 31

Table 1 Patient Characteristics*

	Chemo + Hormone	Chemo	*p*-value
Number of cases	16	12	
Age (Median: mini.-max.)	48.6 (34–62)	49 (35–64)	0.944
T at diagnosis (TNM)			
T1	3	1	0.453
T2	10	11	
T3	1	0	
T4	2	0	
Nodal status at diagnosis			
Negative	8	8	0.459
Positive	8	4	
Histologic grade			
1	1	2	0.687
2	13	8	
3	2	2	
ER status			
positive	16	12	1.000
negative	0	0	
PR status			
positive	10	10	0.401
negative	6	2	
Her2 status			
positive	4	1	0.355
negative	12	11	
Menopus status			
pre	9	7	1.000
post	7	5	

*p-value for categorical value was calculated by Fisher exact test; p-value for continuous value was calculated by Wilcoxon test.

patients were enrolled between April 2007 and March 2011 (14 patients from Chugoku Central Hospital, 13 from Okayama University Hospital, 2 from Hiroshima City Hospital, 1 from Onomichi City Hospital and 1 from Okayama Central Hospital). Patients and disease characteristics are presented in Table 1. There were no significant differences in the patient age, clinical T and N stages, ER, PR, HER2 and menopausal status between the treatment groups. Twenty-eight patients fully completed the protocol-specified therapy. One patient withdrew because of grade 3 interstitial pneumonia, and 2 patients withdrew consent. One patient required a dose reduction of epirubicin. Ultimately, 16 patients (57.1%) received the concurrent therapy, of whom 9 were premenopausal (32.1%) and 7 were postmenopausal (25.0%), while twelve patients (42.9%) received chemotherapy only (7 were premenopausal and 5 were postmenopausal). Following completion of neoadjuvant therapy, all patients proceeded to surgical resection of their breast cancers. Of 14 patients (50.0%) considered ineligible for breast-conserving surgery at baseline, 6 (42.9%) were able to undergo achieved breast-conserving surgery and 8 (57.1%) underwent mastectomy. Of the 6 patients with breast-conserving surgery, 4 (66.7%) received the concurrent therapy and 2 (33.3%) received chemotherapy only. The remaining 14 patients (50.0%) underwent breast-conserving surgery as planned at presentation.

pCR rate after neoadjuvant treatment. First, we assessed pCR rates in 2 groups after neoadjuvant treatment, and there were no significant differences ($p=1.000$) between concurrent chemo-endocrine therapy (pCR rate: 12.5% (=2/16), and chemotherapy alone (8.3% (=1/12)).

Change in tumor size between pre and post neoadjuvant treatment. Second, we assessed overall tumor response by change in tumor size between pre- and post-neoadjuvant therapy. Nine cases (32.1%) showed progressive disease in spite of neoadjuvant therapy, including 5 (31.3%: 5/16) with concurrent therapy and 4 (33.3%: 4/12) with chemotherapy alone. Fig. 1 shows the change in tumor size between pre- and post-therapy in 2 regimens; tumor size after therapy was significantly reduced in the concurrent therapy group ($p=0.035$), but not in the chemotherapy-alone group ($p=0.622$). In the concurrent group, there was a marginally significant tumor response in the high-Ki67 group ($p=0.067$), but not in the low-Ki67 group ($p=0.356$). In the chemotherapy-only group, both Ki67 groups (high and low Ki67 level) had no significant differences in tumor size.

Measurement of histopathological response. Next, we assessed histopathological response by change in IHC-Ki67 level and RCB in the 2 different neoadjuvant regimens. In IHC-Ki67 analyses, 3 cases (10.7%) that achieved pCR were excluded from the analysis, because we could not evaluate the IHC-Ki67 level after neoadjuvant therapy. In the remaining cases a significant decrease in the IHC-Ki67 level was observed between pre-treatment biopsies and excision specimens; however, there was no statistically significant difference between the 2 treatment regimens

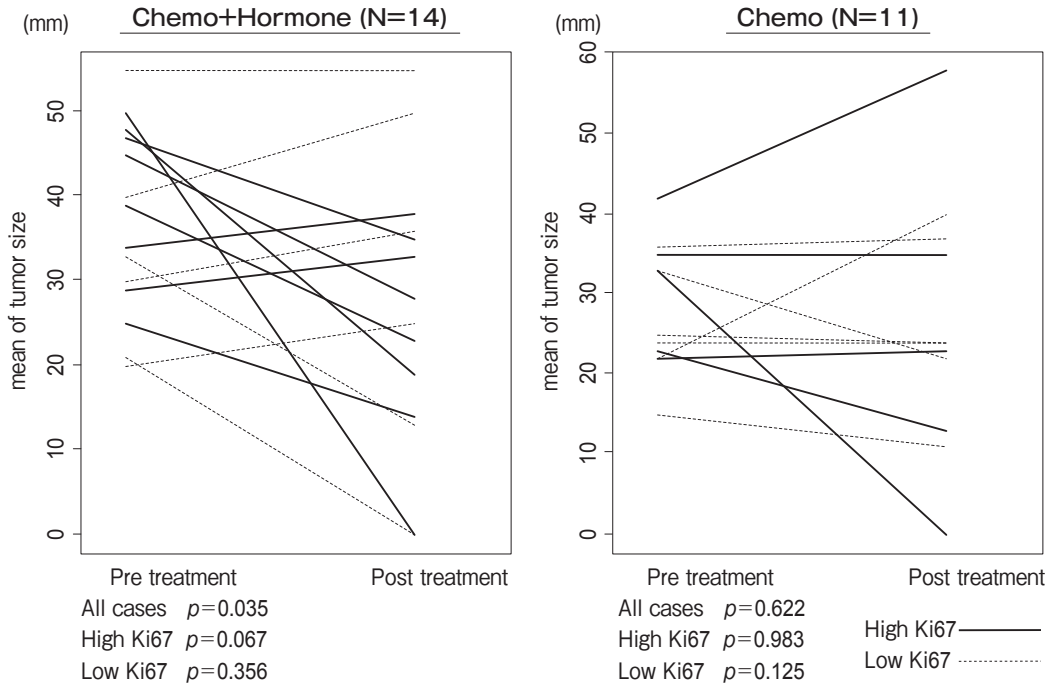

Fig. 1 Two-way interaction plots for tumor size between pre- and post- neoadjuvant therapy. Two-way interaction plots showed change in tumor size between pre- and post-neoadjuvant therapy. *P*-value was calculated by paired match *t*-test.

(Concurrent therapy, p-value=0.005; Chemotherapy only, p-value=0.021, Fig. 2). There were 2 cases (Concurrent group, 1; Chemotherapy group, 1) with a paradoxical increase of IHC-Ki67 level compared with pre-treatment status. In the low-IHC-Ki67 group, the concurrent-therapy group had a significantly decrease in IHC-Ki67 level (p-value=0.003), but the chemotherapy-only group did not (p-value=0.147). On the other hand, in the high-IHC-Ki67 group, both the concurrent-therapy and chemotherapy-only groups had relatively favorable decreases in tumor size (Concurrent: p-value=0.084, Chemo only: p-value=0.026).

Finally, we assessed RCB in the treatment groups. Of the 16 patients who received concurrent therapy, 2 (12.5%) achieved an RCB-0 (pCR), 11 (68.8%) achieved RCB-II, and 3 (18.8%) achieved RCB-III. Of the 12 patients who received chemotherapy only, 1 (8.3%) achieved RCB-0 (pCR), 3 (25.0%) achieved RCB-I, 7 (58.3%) achieved RCB-II, and 1 (8.3%) achieved RCB-III (Table 2). There was no significant difference in the RCB index between the 2 treatment groups (p-value=0.143, Fig. 3). When we assessed RCB by IHC-Ki67 level, there were no significant differences between 2 regimens in either the high- or low-IHC-Ki67 group.

Table 2 RCB class in the treatment groups (N° of Pt.)

RCB class	Chemo+Hormone	Chemo only
0	2	1
I	0	3
II	11	7
III	3	1

Discussion

We examined whether ER-positive breast cancer patients treated by concurrent administration of chemotherapy plus an LHRH agonist or an AI benefit differently from those receiving chemotherapy alone in the neoadjuvant setting. pCR rates after neoadjuvant therapy in the 2 groups were not significantly different. Previous studies showed that the expected rate for pCR after neoadjuvant chemotherapy in

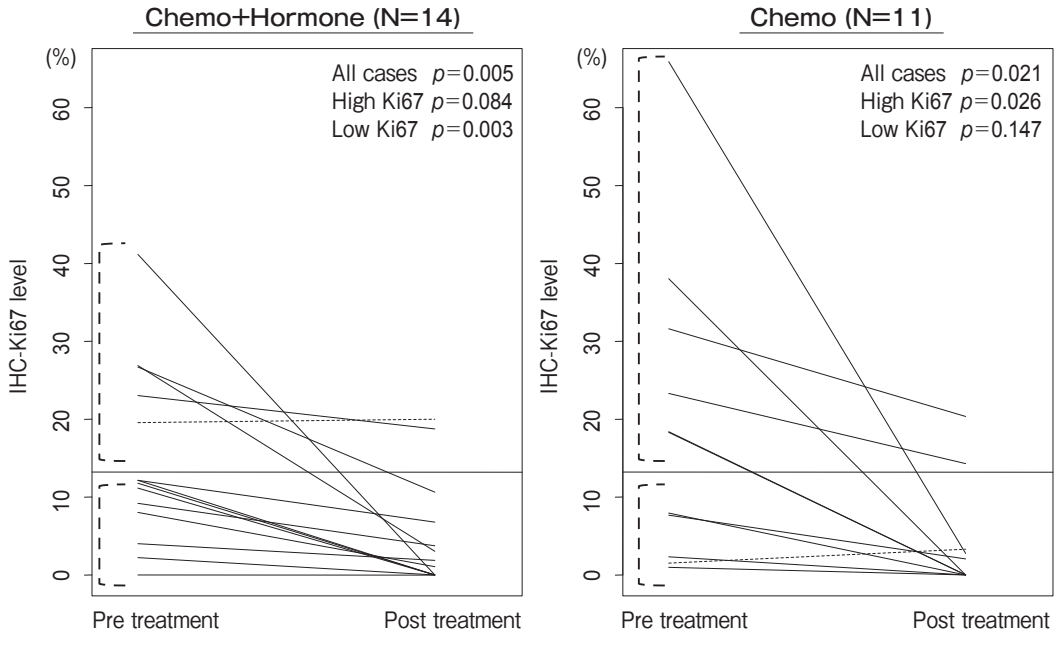

Fig. 2 Two-way interaction plots for IHC-Ki67 level between pre- and post- neoadjuvant therapy. Two-way interaction plots show the IHC-Ki67 level between pre- and post-neoadjuvant chemotherapy. The *p*-value was calculated by a paired *t*-test. The broken line indicates cases with paradoxical increase of IHC-Ki67 level between pre- and post-treatment; horizontal line (13.25%) indicates the cutoff between high and low IHC-Ki67 level.

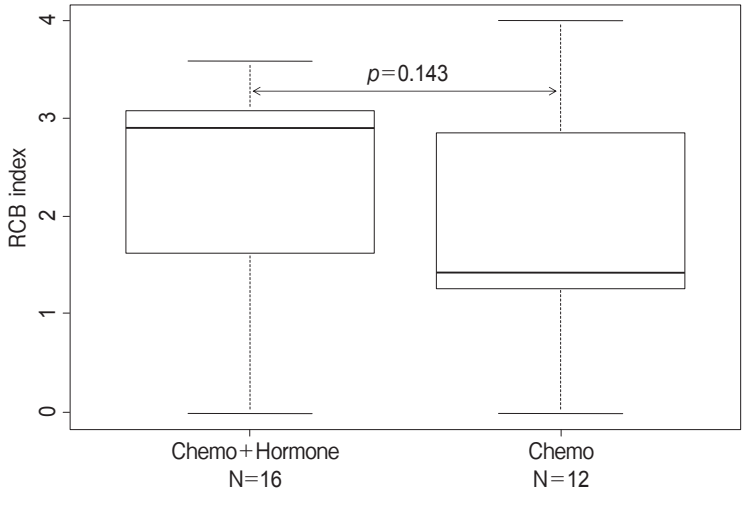

Fig. 3 Box plots for RCB index according to neoadjuvant therapy regimens. Box plots show the RCB index level in 2 different regimens (concurrent chemo-endocrine therapy and chemotherapy only). *P*-values were calculated by the Wilcoxon test.

ER-positive entities is lower than in ER-negative ones, and that ER-positive histology is a relative contraindication for neoadjuvant chemotherapy because the expected benefit is modest because of less frequent clinical response [2–6]. In contrast to chemotherapy, neoadjuvant endocrine therapy is well tolerated, with very few patients having to discontinue the treatment because of side effects. However, the pCR rate for those given neoadjuvant endocrine therapy is not necessarily satisfactory [8, 12]. In the preclinical level, Sui *et al.* [24] showed that the combination of antiestrogen agents such as fulvestrant might be a strategy to reverse ER-mediated chemoresistance or sensitize ER-positive breast tumors to chemotherapeutic

agents. Moreover, Ikeda *et al.* [22] also reported that combination treatment with fulvestrant and various cytotoxic agents (paclitaxel, docetaxel, vinorelbine, and 5-fluorouracil) has a synergistic effect in ER-positive breast tumors. Regarding chemoresistant factors, Bcl-2 and microtubule-associated protein tau were downregulated by fulvestrant. These studies used as the anti-estrogen agent fulvestrant, which specifically binds to and destroys the ER, blocking all estrogen activity, unlike tamoxifen. Unlike the preclinical data, we observed no significant difference in the pCR rate for ER-positive breast cancers treated by concurrent chemo-endocrine therapy with exemestane or an LHRH analogue, and chemotherapy alone, indicating that giving concurrent therapy for ER-positive breast cancers in the neoadjuvant setting might not be as effective as chemotherapy alone for achieving pCR.

One of the reasons that our study did not show significant benefits from concurrent therapy might be the heterogeneity within ER-positive and HER2-negative breast cancers [31]. ER-positive breast cancers do not benefit equally from chemotherapy. In past studies, ER-positive tumors with high proliferation have shown a large chemotherapy benefit, while those with low and intermediate proliferation derived minimal, if any, benefit from chemotherapy treatment [32]. Vaile *et al.* also reported the decreasing benefit of chemotherapy with increasing ER expression, indicating the inadequacy of chemotherapy alone for patients with higher ER expression [33]. As in previous reports, we showed that cases with high but not low Ki67 had significant reduction in Ki67 level from chemotherapy alone (p-value = 0.026), implying that ER-positive cases have distinct chemotherapy sensitivity according to Ki67 level, and that we should select neoadjuvant regimens based on not only clinical stage, but also tumor characteristics. More recent results indicate that Ki67 level might represent a valid surrogate of outcome in patients with ER-positive breast cancer treated with neoadjuvant endocrine therapy. In fact, tumor Ki67 levels determined during neoadjuvant endocrine treatment were found to be a marker of treatment efficacy and to have a substantial prognostic value [34, 35]. In these studies, reductions of Ki67 level may represent good efficacy from chemotherapy and/or hormone therapy and favorable clinical course. Our findings confirmed similar significant reduction of Ki67 level in both regimens (Concurrent: p-value = 0.005, Chemotherapy alone: p-value = 0.021), indicating that patients have similar favorable clinical outcomes in both regimens, and the effect of adding short-term hormone therapy on breast cancer prognosis may be limited. Distinct subtypes of breast cancer may need distinct types of strategies. The other possibility might come from the small sample size. We could collect only 28 cases, although we estimated a need for 120 cases to arrive at significant differences between the 2 groups. In future trials, more precise estimations and better organization will be needed.

Next, we assessed residual disease after neoadjuvant therapy in order to improve the prognostic information that can be obtained from evaluating pathologic response. Symmans *et al.* [28] showed that RCB after neoadjuvant chemotherapy was independently prognostic in a multivariate model that included various clinical and pathological covariates. We showed similar RCB distributions in 2 distinct regimens, indicating again that administering concurrent hormone therapy of only short duration may not serve to improve breast cancer prognosis [36]. We also confirmed the poor correlations between IHC-Ki67 level and RCB index (Spearman's rank correlation rho = 0.116, data not shown). The Ki67 level identifies cells in the G1/S and M phases of the cell cycle [37]; changes in Ki67 value, indirectly assessing cell proliferation, may be more appropriate at predicting the risk of recurrence in breast cancers, as well to predict the magnitude of the chemotherapy benefit in ER-positive breast cancers [32, 38] than the pCR rate, which directly assesses carcinoma cell number and cellularity. On the other hand, RCB provides prognostic information from the primary tumor bed area, overall cancer cellularity, the percentage of cancer that is *in situ* disease, lymph node infiltration, and diameter of largest metastasis [28]. By taking into consideration a broad range of actual responses from near pCR to outright treatment resistance, RCB can identify patients with resistant disease more precisely than dichotomization of responses into pCR or RD. The 2 biomarkers may be similar, but they have different predictive power for prognosis and provide different prognostic information.

Our study has a number of limitations; primarily, the number of patients enrolled is too small. Furthermore, the adequacy of an LHRH agonist in

suppressing ovarian function in premenopausal patients is uncertain. It is also uncertain whether surrogate markers including IHC-Ki67 and RCB yield proper prognostic information after concurrent neoadjuvant chemo-endocrine therapy. These could give rise to false discovery.

Based on our findings, the concurrent chemo-endocrine therapy did not provide significant differences in pCR rates, but provided significant reduction of tumor size after neoadjuvant therapy in breast cancer patients with hormone receptor-positivity compared to chemotherapy alone.

Based on our findings, we cannot recommend routine administration of concurrent chemo-endocrine therapy in the clinical neoadjuvant setting. A small subpopulation in ER-positive and HER2-negative cases may be suitable for concurrent therapy, although we could not reveal it or identify any biomarkers to stratify. These preliminary results should be followed up by further studies; however, the neoadjuvant concurrent chemo-endocrine therapy approach should still be considered as investigational and should not be used outside a clinical trial.

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