

cannot confirm the efficacy by adjuvant chemotherapy itself. However, non-sensitive patients have to endure relatively needless therapy for about 6 months, so it is very important to make the pre-diagnosis of sensitivity to chemotherapy if possible. Several biological markers that might predict response are under investigation [1-9]. Estrogen receptor, progesterone receptor, and HER2 are very useful markers for the selection of anticancer drugs and prediction of prognosis, but are not useful for predicting the response to chemotherapeutic agents such as anthracycline and taxane. Therefore, other biological markers from pre-treatment core needle biopsy are needed to predict the response to NAC, to individualize treatment, and to achieve maximal efficacy.

In this study, we investigated biological markers from pre-treatment core needle biopsies of highly sensitive tumors and non-sensitive tumors and identified additional prognostic markers that might predict the response to NAC and aid in the selection of treatment strategy.

Materials and Methods

All patients with operable breast cancer who were treated between May 1998 and July 2006 at the National Cancer Center Hospital with anthracycline and/or taxane as NAC were included in this retrospective study. NAC was indicated for clinical stage II breast cancer patients with tumors larger than 3 cm and stage III breast cancer patients. Core needle biopsy was performed before NAC to allow pathological diagnosis. Doxorubicin (DOX, 50 mg/m²) and docetaxel (DTX, 60 mg/m²) were administered for four 3-week cycles before surgery. Additional adjuvant treatment with DOX/DTX was given if patients achieved complete or partial remission after NAC. Otherwise, patients were treated with four cycles of iv cyclophosphamide, methotrexate, and 5FU. Trastuzumab was not administered to the patients with HER2-overexpressing tumors. Tamoxifen (20 mg/day) or anastrozole (10 mg/day) was administered for 5 years after surgery if either the pretreatment biopsy specimen or the surgical specimen post-chemotherapy was positive for estrogen-receptor or progesterone receptor.

Pretreatment diagnosis was established by our pathologists using samples from core needle biopsy or

surgical resection. Overexpression of hormone receptors, p53, HER2 and EGFR was examined by immunohistology. Surgical specimens were sectioned at about 7-10 mm and classified for pathological response. Pathological features were described and invasive ductal carcinomas were classified into 3 subtypes (papillotubular, solid tubular, and scirrhous) according to the General and Pathological Recording of Breast Cancer guidelines established by the Japanese Breast Cancer Society [10]. The criteria for histological grading of IDC were based on a modification of those recommended by the WHO [11, 12]. The response criteria used in this study include Fisher's system [13], complete pCR denotes no histological evidence of tumor cells, pCR with DCIS denotes no histological evidence of invasive tumor cells (specimens with only noninvasive cells included), and pINV denotes the presence of invasive tumor cells. Overexpression of ER (1D5, Dako Cytomation, Baltimore, MD, USA), PgR (1A6, Novocastra), HER2 (Herceptest, Dako), p53 (DO7, Dako), and EGFR (2-18C9, Dako) were examined by immunohistology using the noted antibodies. The criterion for ER, PgR, and p53 was staining of more than 10% of cancer cell nuclei, regardless of intensity. HER2 and EGFR grading is as follows: 0: negative, 1+: slightly positive in more than 10% of cancer cells, 2+: moderately positive in more than 10% of cancer cells, 3+: markedly positive in more than 10% of cancer cells. 2+ and 3+ were considered positive for HER2 and EGFR.

Clinical response to NAC was decided from the 2 greatest perpendicular diameters (before each chemotherapy treatment and before surgery) of tumors in the breast and axillary lymph nodes. Absence of clinical evidence of palpable tumors in the breast and axillary lymph nodes was defined as a clinical complete response (cCR). Reduction in total tumor size of 30% or greater was graded as clinical partial response (cPR). An increase in total tumor size of more than 20% or appearance of new suspicious ipsilateral axillary adenopathy was considered progressive disease (cPD). Tumors that did not meet the criteria for objective response or progression were classified as stable disease (cSD). In this study, we analyzed biological markers from core needle biopsies before NAC in complete pCR cases and non-sensitive tumors (clinical SD and PD), and demonstrated bio-

logical predictors of pathological response to PST.

Statistical analysis was carried out using JMP version 6.0 (SAS Institute Inc., Cary, NC, USA). Associations between ordinal variables were assessed using χ^2 analyses or the Fisher exact test for two-by-two variables. The statistical significance (P) was taken as a measure of the strength of evidence against the null hypothesis, and $p \leq .05$ was considered statistically significant.

Results

One hundred and eighty-three patients with operable breast cancer were treated with NAC at National Cancer Center Hospital between May 1998 and October 2001. Table 1 lists the patient and tumor characteristics. The median age was 50 years (range: 29–70). At diagnosis, 41 (22%) patients were in stage IIA, 63 (34%) were in stage IIB, 37 (20%) were in stage IIIA, and 42 (23%) were in stage IIIB. Breast conserving surgery was performed for 55 (30%) patients after NAC. The overall clinical response rate

to NAC was 83% (cCR + cPR) and the pCR rate was 13%. 30 (17%) patients were insensitive to NAC (cSD or cPD). Among the responsive patients, 9 (5%) exhibited complete pCR (pathologically no tumor in the breast) and 14 (8%) exhibited pCR with DCIS.

Immunohistological characteristics from core needle biopsy before NAC are listed in Table 2. There were 62 (34%) cases of solid tubular primary tumor, 65 (36%) scirrhous, 34 (19%) papillotubular, 9 (5%) ILC, and 3 (2%) mucinous carcinomas. 88 (48%) cases were histological grade 3. 66 (36%) were ER positive and 72 (39%) were PgR positive. 73 (40%) were HER-2 positive (2+ and 3+ in immunohistological examination).

We evaluated age, histological type, histological grade, ER, PgR, HER2, EGFR, and p53 as predictive factors for response to NAC by comparing 9 (5%) sensitive (complete pCR) and 30 (17%) insensitive (cSD and cPD) tumors (Table 3). In univariate analysis, histological grade 3 ($p = 0.011$) and p53 ($p = 0.045$) were significant predictors of complete pCR. However, EGFR and HER2 were not predic-

Table 1 Patient and tumor characteristics

Parameter	No. of patients (%)
Total	183
Age (median)	50 (29–70)
Clinical stage	
Stage IIA	41 (22%)
Stage IIB	63 (34%)
Stage IIIA	37 (20%)
Stage IIIB	42 (23%)
Operation	
Bt + Ax	128 (70%)
Bp + Ax	55 (30%)
Clinical response	
cCR	32 (17%)
cPR	121 (66%)
cNC	29 (16%)
cPD	1 (1%)
Pathological response	
complete pCR	9 (5%)
pCR with DCIS	14 (8%)
pINV	160 (87%)

Bt, total mastectomy; Bp, partial mastectomy; Ax, axillary lymph node dissection.

Table 2 Immunohistological characteristics of CNB before PST

Parameter	No. of patients (%)
Histological type	
IDC	161 (88)
Solid tubular	62 (34)
Scirrhous	65 (36)
Papillotubular	34 (19)
ILC	9 (5)
mucinous	3 (2)
others	10 (5)
Histological grade	
3	88 (48)
2	88 (48)
1	7 (4)
ER	
positive	66 (36)
negative	117 (64)
PgR	
positive	72 (39)
negative	111 (61)
HER2	
positive (2 + and 3 +)	73 (40)

tors.

We analyzed the immunohistological features of CNB specimens. The correspondence rates of these features in insensitive patients between CNB and surgical specimens are shown in Table 4. The correspondence rates for histological type, histological grade 3, ER, PgR, HER2, p53, and EGFR were 70%, 73%, 67%, 70%, 80%, 93%, and 73%. The correspondence rate of EGFR was not low; however, in almost all patients with a discrepancy between CNB and surgical specimens, EGFR overexpression changed from negative to positive.

Discussion

The identification of predictive factors for NAC is very important for order made cancer treatment. The development of new medicines has diversified chemotherapeutic regimens, and the selection of treatment strategy according to individual cancer characteristics has become more difficult. To aid in selection, translational research has begun to demonstrate important correlations between prognostic factors and sensitivity to chemotherapy.

In this study, we retrospectively evaluated response to NAC including anthracycline and taxane and a number of biomarkers. We found that pathologic response significantly associated with p53 expression and histological grade 3.

In our analysis, p53 could predict response of NAC. p53 accumulation was reported to be associated with a poor response to anthracycline in node-negative breast cancer patients [14], and may compromise the efficacy of anthracycline but not of taxane [15]. All patients in this study received both anthracycline and taxane, and p53 was an independent predictive factor of response to NAC similar to these reports. We cannot analyze the response of anthracycline and taxane respectively. However commonly we use both drugs in NAC. If the tumor has p53 mutation before NAC, we should check the response of anthracycline tightly and change to taxane when the response is wrong.

Previous studies reported poor prognosis for patients with HER2-overexpression. Several studies indicate that HER2 expression can predict sensitivity to anthracycline chemotherapy [16]; however, in this study, HER2 was not a predictor of pCR to NAC. HER2 negative patients rate were 22% of good responders and 33% of poor responders. In this study trastuzumab was not administered to patients with HER2 overexpression tumors. However, in these days, trastuzumab significantly improved the prognosis and the response to chemotherapy in these patients [17]. It was reported that the rate of pCR patients administered trastuzumab was significantly high. HER2 expression was not predictor of response to anthracycline and taxane in this study. We need to examine the relationship between HER2-overexpression and response to chemotherapy with trastuzumab.

Table 4 Correspondence rates of biological markers in insensitive patients between CNB and surgical specimens

Parameter	%
Histological type	70
Histological grade 3	73
ER	67
PgR	70
HER2	80
p53	93
EGFR	73

Table 3 Univariate analysis of clinicopathological features between sensitive (pCR) and insensitive cases (cNC + cPD)

Parameter	Sensitive (n = 9) (%)	Non-sensitive (n = 30) (%)	p-value
Age < 50	3 (33)	19 (63)	N.S.
Histological type (so.)	6 (67)	12 (40)	N.S.
Histological grade 3	8 (89)	13 (43)	0.011
ER negative	8 (89)	17 (57)	N.S.
PgR negative	6 (67)	17 (57)	N.S.
HER2 positive	2 (22)	10 (33)	N.S.
p53 positive	5 (56)	6 (20)	0.045
EGFR positive	3 (33)	7 (23)	N.S.

so, solid tubular carcinoma

A previous study observed EGFR expression in 37–80% of basal-like tumors, as identified by DNA microarray, and reported poorer prognosis for this phenotype [18–20]. We hypothesized that EGFR expression might distinguish the basal-like phenotype and predict poorer response to NAC. However, in this study, EGFR was not an independent predictive factor of response to NAC. It was reported that EGFR is expressed in 7–36% of breast carcinomas with high grade conventional invasive ductal carcinoma (IDC) [21–24] and EGFR expression was seen in 272 (20%) of 1388 cases. In a univariate analysis, Tsutsui *et al.* showed a significantly poorer clinical outcome for patients with EGFR-positive tumors compared with those who were EGFR-negative, both for overall survival and disease-free survival [21]. The correspondence rate of EGFR overexpression between core needle biopsy and surgical specimens was higher than the correspondence rates of common predictive factors (ER, PgR, and HER2) between the 2 types of specimens. However, the rates of EGFR expression were relatively low in both sensitive (33%) and insensitive patients (23%). In addition, in cases in which EGFR expression did not correspond between CNB and surgical specimens, EGFR was always negative in CNB, but positive in the surgical specimen. Therefore, it is possible that core needle biopsy specimens are inadequate to evaluate EGFR overexpression, or that EGFR expression was stimulated by chemotherapy. Following NAC, highly malignant EGFR-positive tumor cells increased in number, while EGFR-negative cells decreased in number. In these specimens, other common predictive factors did not change pre- and post-NAC; therefore it is not certain that all of the CNB specimens were inadequate. Indeed, it may be that NAC changed the characteristics of some tumors.

We evaluated EGFR, HER2, p53 and other common markers in specimens from pretreatment core needle biopsies as predictors of response to NAC. p53 was a more significant predictor than ER and histological grade, factors that have been previously reported. These results may have been influenced by the uncertainty of core needle biopsy results and the heterogeneity of cancer cells in the tumors. The correspondence rates of these common markers between CNB and surgical specimens were relatively low. However, the correspondence rate of p53 was signifi-

cantly high. This result indicates that p53 is a stable parameter and suitable for predicting the response to neoadjuvant chemotherapy and for pretreatment diagnosis from CNB specimens.

Pretreatment diagnosis from CNB specimens is necessary to decide the strategy for primary breast cancer treatment. Therefore, identifying prognostic factors is very important, and we need a greater sample size to establish a classification system to predict patient outcome.

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Analysis of Ki-67 Expression With Neoadjuvant Anastrozole or Tamoxifen in Patients Receiving Goserelin for Premenopausal Breast Cancer

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BACKGROUND: The increasing costs associated with large-scale adjuvant trials mean that the prognostic value of biologic markers is increasingly important. The expression of nuclear antigen Ki-67, a marker of cell proliferation, has been correlated with treatment efficacy and is being investigated for its value as a predictive marker of therapeutic response. In the current study, the authors explored correlations between Ki-67 expression and tumor response, estrogen receptor (ER) status, progesterone receptor (PgR) status, and histopathologic response from the STAGE study (Study of Tamoxifen or Arimidex, combined with Goserelin acetate to compare Efficacy and safety). **METHODS:** In a phase 3, double-blind, randomized trial (National Clinical Trials identifier NCT00605267), premenopausal women with ER-positive, early stage breast cancer received either anastrozole plus goserelin or tamoxifen plus goserelin for 24 weeks before surgery. The Ki-67 index, hormone receptor (ER and PgR) status, and histopathologic responses were determined from histopathologic samples that were obtained from core-needle biopsies at baseline and at surgery. Tumor response was determined by using magnetic resonance imaging or computed tomography. **RESULTS:** In total, 197 patients were randomized to receive either anastrozole plus goserelin (n = 98) or tamoxifen plus goserelin (n = 99). The best overall tumor response was better for the anastrozole group compared with the tamoxifen group both among patients who had a baseline Ki-67 index $\geq 20\%$ and among those who had a baseline Ki-67 index $< 20\%$. There was no apparent correlation between baseline ER status and the Ki-67 index in either group. Positive PgR status was reduced from baseline to week 24 in the anastrozole group. **CONCLUSIONS:** In premenopausal women with ER-positive breast cancer, anastrozole produced a greater best overall tumor response compared with tamoxifen regardless of the baseline Ki-67 index. *Cancer* 2013;119:704-13. © 2012 American Cancer Society.

KEYWORDS: anastrozole, aromatase inhibitor, biomarker, neoadjuvant, Ki-67, premenopausal breast cancer.

INTRODUCTION

In addition to ablative surgery, radiotherapy, and cytotoxic chemotherapy, an additional standard treatment option for premenopausal women with estrogen receptor (ER)-positive breast cancer is the ER antagonist tamoxifen, either alone or in combination with ovarian function suppression.¹ Temporary and potentially reversible ovarian suppression can be achieved by treatment with a luteinizing hormone-releasing hormone analog, such as goserelin. Goserelin in combination with tamoxifen has demonstrated improved progression-free survival and disease-free survival compared with goserelin alone in premenopausal women with hormone receptor-positive (ER-positive and/or progesterone receptor [PgR]-positive) breast cancer in the advanced² and adjuvant³ settings.

Nonsteroidal aromatase inhibitors (AIs), including anastrozole and letrozole, and the irreversible steroidal aromatase inactivator exemestane have demonstrated improved efficacy compared with tamoxifen in the advanced⁴⁻⁷ and adjuvant⁸⁻

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¹² treatment settings. Therefore, AIs in combination with ovarian suppression have been evaluated for the treatment of premenopausal women with ER-positive breast cancer.^{13,14}

Neoadjuvant treatment for breast cancer provides an opportunity for downstaging of large tumors to allow patients to undergo breast-conserving surgery rather than mastectomy. Chemotherapy can offer an effective neoadjuvant treatment; however, increasing evidence suggests that ER-positive tumors are less sensitive to chemotherapy.¹⁵ It has been demonstrated that neoadjuvant endocrine therapy has efficacy in the treatment of ER-positive disease among postmenopausal women, resulting in similar objective response rates and rates of breast-conserving surgery for AIs compared with more cytotoxic chemotherapy.¹⁶ Therefore, the role of neoadjuvant endocrine therapy in premenopausal women is also of interest.

With the increasing costs associated with large-scale adjuvant trials, both the prognostic value of biologic markers and the long-term predictive value of short-term trials are increasingly important. The expression of nuclear antigen Ki-67, a marker of cell proliferation, reportedly has been correlated with treatment efficacy and is being investigated for its value as a predictive marker of therapeutic response.¹⁷ In a cross-trial comparison, an increased reduction in Ki-67 expression after neoadjuvant treatment with anastrozole compared with tamoxifen was observed consistently; and increased progression-free survival has been reported for anastrozole versus tamoxifen in the adjuvant Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial.^{8,18,19}

The STAGE study (Study of Tamoxifen or Arimidex Combined With Goserelin Acetate to Compare Efficacy and Safety) was the first randomized trial to compare anastrozole plus goserelin versus tamoxifen plus goserelin in the neoadjuvant setting (24 weeks of therapy) in premenopausal women with ER-positive and human epidermal growth factor receptor 2 (HER2)-negative, operable breast cancer. The patients who received anastrozole plus goserelin in that trial had a superior best overall tumor response compared with the patients who received tamoxifen plus goserelin, as measured on magnetic resonance imaging (MRI) or computed tomography (CT) studies (anastrozole plus goserelin, 64.3%; tamoxifen plus goserelin, 37.4%; estimated difference, 26.9%; 95% confidence interval [CI], 13.5-40.4; $P < .001$). The treatment effect was consistently in favor of anastrozole, regardless of the measurement methods (caliper and ultrasound). The histopathologic response rate also was better in the anastrozole group (anastrozole plus goserelin, 41.8%; tamoxifen plus goserelin, 27.3%; estimated difference, 14.6%; 95%

CI, 1.4-27.7; $P = .032$). Both treatment regimens were well tolerated, consistent with the known safety profiles of anastrozole, tamoxifen, and goserelin.²⁰ The geometric mean Ki-67 index at baseline was 21.9% in the anastrozole group and 21.6% in the tamoxifen group. At week 24, the Ki-67 index was reduced in both treatment groups (to 2.9% in the anastrozole group and to 8% in the tamoxifen group). The reduction from baseline to week 24 was significantly greater with anastrozole than with tamoxifen. The estimated ratio of reduction between groups was 0.35 (95% CI, 0.24-0.51; $P < .001$).²⁰ Here, we report an exploratory analysis of the STAGE study that investigated potential correlations between the Ki-67 index and the best overall tumor response, ER status, PgR status, or histopathologic response.

MATERIALS AND METHODS

Study Design and Patients

In this phase 3, double-blind, randomized, parallel-group, multicenter trial, the participating patients were premenopausal women ≥ 20 years with ER-positive and HER2-negative breast cancer who had operable and measurable lesions (tumors measuring 2-5 cm, negative lymph node status [N0], and no metastases [M0]). Inclusion and exclusion criteria have been described previously.²⁰

Patients were randomized 1:1 to receive either oral anastrozole 1 mg daily with a tamoxifen placebo or oral tamoxifen 20 mg daily with an anastrozole placebo. Both treatment groups received goserelin 3.6 mg as a subcutaneous injection every 28 days. Treatment continued for 24 weeks before surgery or until patients met any criterion for discontinuation.

The primary study endpoint was the best overall tumor response during the 24-week neoadjuvant treatment period. Secondary endpoints included histopathologic response, changes in estrone (E_1) and estradiol (E_2) serum and breast tumor tissue concentrations, changes in Ki-67 expression, and tolerability. For this exploratory analysis, we assessed correlations between Ki-67 expression and tumor response, ER status, PgR status, or histopathologic response.

The protocol was approved by an institutional review board at all study sites, and all enrolled patients provided written informed consent. The study (National Clinical Trials identifier NCT00605267) was conducted in accordance with the Declaration of Helsinki and good clinical practice, the applicable local regulatory requirements, and the AstraZeneca policy on Bioethics.

Assessments

Tumor measurements were performed using caliper measurements, ultrasound, or MRI or CT studies. The

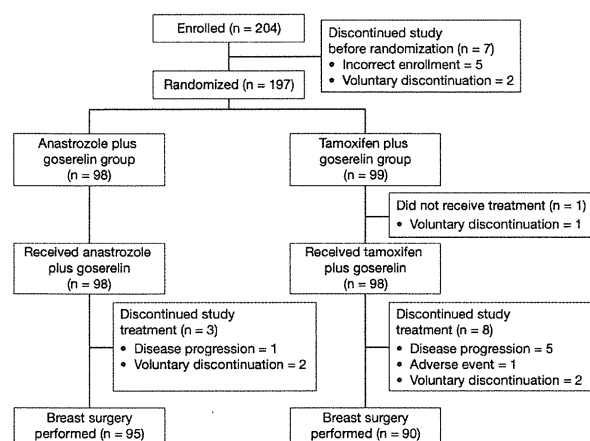


Figure 1. This is a CONSORT (Consolidated Standards of Reporting Trials) diagram of the current study.

primary analysis indicated that the best overall tumor response for anastrozole versus tamoxifen was consistent, regardless of the measurement method used.²⁰ We present tumor response data from the MRI or CT measurements at day 0 and at 24 weeks. The objective tumor response was assessed according to modified Response Evaluation Criteria in Solid Tumors (RECIST).²¹

The status of Ki-67, ER, and PgR was determined using histopathologic core-needle biopsy specimens that were collected at baseline and at surgery. Tissue sections were fixed in formalin and stored at room temperature before immunohistochemical staining. Ki-67 expression was determined by staining sections with an anti-MIB-1 antibody at a central laboratory (SRL Inc., Tokyo, Japan) for assessment by a central review board. For all slides, photomicrographs were taken from 3 to 5 hotspots at $\times 20$ magnification using light microscopy. Two pathologists independently assessed the photomicrographs, and the Ki-67 index was calculated as the ratio of Ki-67-positive cancer cells from a total of 1000 cancer cells. ER-positive status and PgR-positive status at baseline were defined as $\geq 10\%$ staining of cancer cell nuclei determined by a pathologist at each individual study site (nuclei were assessed using mouse monoclonal antibody clones 6F11 and 16, respectively). Staining for ER and PgR also was assessed in parallel using Allred scores by the Central Pathologist Review Committee.²² An Allred score (the proportion score plus the intensity score) of ≥ 3 defined ER or PgR positivity, a score from ≥ 3 to < 7 indicated medium expression, and a score of ≥ 7 indicated rich expression.

Histopathologic effects were assessed by comparing histopathologic samples that were obtained at baseline and at surgery. For the assessment of histopathologic

response, the following categories were used: grade 0 indicated no response; grade 1a, marked change in < 1 of 3 cancer cells; grade 1b, marked changes in ≥ 1 of 3 but < 2 of 3 cancer cells; grade 2, marked changes in ≥ 2 of 3 cancer cells; and grade 3, necrosis or disappearance of all cancer cells and replacement of all cancer cells by granuloma-like and/or fibrous tissue. The histopathologic response was defined as the proportion of patients whose tumors were classified as grade 1b, 2, or 3.^{23,24}

Post hoc subset analyses were used to determine correlations between the baseline Ki-67 index ($\geq 20\%$ vs $< 20\%$) and the best overall tumor response. The percentage change in the Ki-67 index for responders (patients whose best overall tumor response was a complete or partial response) versus nonresponders (patients whose best overall tumor response was stable or progressive disease) also was compared. Correlations between the baseline Ki-67 index and the histopathologic response at week 24 also were evaluated, and we used post hoc analyses to investigate correlations between changes in the Ki-67 index from baseline to week 24 and ER or PgR status at baseline. Positive ER and PgR status (Allred score ≥ 3) also was assessed at baseline and at week 24. Preoperative Endocrine Prognostic Index (PEPI) scores, which were calculated post hoc as the sum of risk points weighted by the size of the hazard ratio for tumor size, pathologic lymph node status, ER status, and Ki-67 expression for both recurrence-free and breast cancer-specific survival, were determined for each patient at surgery according to the methods described by Ellis and colleagues.²⁵

Statistical Analysis

The sample size calculation and the main statistical analyses have been described previously.²⁰ All randomized patients were included in the intent-to-treat analysis set.

In a post hoc exploratory analysis, chi-square tests were performed to compare the best overall tumor response at week 24 between baseline Ki-67 index categories ($\geq 20\%$ vs $< 20\%$) within each treatment group and between treatment groups within each baseline Ki-67 index category. A chi-square test also was used to compare the histopathologic response at 24 weeks between the baseline Ki-67 index categories within each treatment group. All tests were made at the nominal 2-sided significance level of .05.

RESULTS

Patients

In total, 197 patients were randomized to receive either anastrozole plus goserelin ($n = 98$) or tamoxifen plus goserelin ($n = 99$) (Fig. 1). Patient demographics and

TABLE 1. Patient Demographics and Baseline Tumor Characteristics

Characteristic	No. of Patients (%)	
	Anastrozole Plus Goserelin	Tamoxifen Plus Goserelin
No. of patients	98	99
Age: Median [range]	44 [28-54]	44 [30-53]
Body mass index: Mean \pm SD, kg/m ²	22.2 \pm 3.5	22.1 \pm 3.3
Histology type		
Infiltrating ductal carcinoma	87 (88.8)	91 (91.9)
Infiltrating lobular carcinoma	3 (3.1)	3 (3)
Other ^a	8 (8.2)	5 (5.1)
Tumor grade		
1	42 (42.9)	48 (48.5)
2	36 (36.7)	26 (26.3)
3	4 (4.1)	14 (14.1)
Not assessable	1 (1)	0 (0)
Not done	15 (15.3)	11 (11.1)
Hormone receptor status		
ER positive	98 (100)	99 (100)
PgR positive	93 (94.9)	87 (87.9)
HER2 negative	98 (100)	99 (100)

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PgR, progesterone receptor; SD, standard deviation.

^aOther included adenocarcinoma (n = 3).

baseline characteristics generally were well balanced between the treatment groups (Table 1). Paired samples for calculating changes in the Ki-67 index from baseline to week 24 were available for 89 patients in the anastrozole plus goserelin group and for 86 patients in the tamoxifen plus goserelin group.

Correlation of the Baseline Ki-67 Index and Best Overall Tumor Response

With a mean baseline Ki-67 index of 21.9% and 21.6% in the anastrozole and tamoxifen treatment groups, respectively, we used post hoc subset analyses to compare patients according to their baseline Ki-67 index (≥ 20 vs $< 20\%$). For anastrozole versus tamoxifen, best overall tumor response from baseline to week 24 was better with anastrozole plus goserelin versus tamoxifen plus goserelin both in patients who had a baseline Ki-67 index $\geq 20\%$ (73.2% vs 44.8%; $P = .002$) and in patients who had a baseline Ki-67 index $< 20\%$ (52.5% vs 29%; $P = .035$) (Fig. 2A).

Within the treatment groups, the best overall tumor response from baseline to 24 weeks, as measured by MRI or CT, was significantly better with anastrozole plus goserelin for patients who had a baseline Ki-67 index $\geq 20\%$ than for those who had a baseline Ki-67 index $< 20\%$ (73.2% vs 52.5%; $P = .036$). Among patients in the tamoxifen plus goserelin group, the best overall tumor response was 44.8% for patients who had a baseline Ki-67

index $\geq 20\%$ and 29% for those who had a baseline Ki-67 index $< 20\%$ ($P = .118$) (Fig. 2A).

Correlation of the Baseline Ki-67 Index and Histopathologic Response

There was no significant difference in the histopathologic response between patients who had a baseline Ki-67 index $\geq 20\%$ versus patients who had a baseline Ki-67 index $< 20\%$ in either treatment group (Fig. 2B).

Correlation of Change in the Ki-67 Index and Responders/Nonresponders

A waterfall plot of changes in the Ki-67 index for individual patients, illustrated according to responders or nonresponders, is provided in Figure 3. There was no apparent relation between a change in Ki-67 expression from baseline to week 24 for responders and nonresponders in either treatment group.

Correlation of the Baseline Ki-67 Index and Estrogen Receptor or Progesterone Receptor Status

In both treatment groups, positive ER status, as determined by the Allred score, was observed in 100% of patients at baseline and at week 24, and $> 90\%$ of patients in both treatment groups were ER rich (baseline Allred score, ≥ 7). Therefore, it was not possible to determine any potential relation between the baseline ER Allred score and the percentage change in Ki-67 expression from baseline to week 24 in either treatment group.

In the anastrozole plus goserelin group, 98.9% of patients were positive for PgR expression at baseline, and 34.4% were positive for PgR expression at week 24. The percentage of patients with positive PgR status was not altered from baseline (91.9%) to week 24 (89.5%) in the tamoxifen plus goserelin group (Fig. 4A). In both treatment groups, the mean decrease in the Ki-67 index was greater in patients who had a baseline PgR Allred score ≥ 7 (anastrozole group, -88.8% ; tamoxifen group, -67.6%), compared with patients who had a baseline PgR Allred score < 7 (anastrozole group, -74.1% ; tamoxifen group, -32.8%) (Fig. 4B).

Preoperative Endocrine Prognostic Index Score

In the anastrozole treatment group, 33.3% of patients had a PEPI score of 0 compared with 11.4% in the tamoxifen group. Fewer patients (21.4%) had a PEPI score ≥ 4 in the anastrozole group compared with patients in the tamoxifen group (36.7%; $P = .002$) (Table 2).

DISCUSSION

In this exploratory analysis, we investigated changes in Ki-67 expression among patients from the STAGE study, a

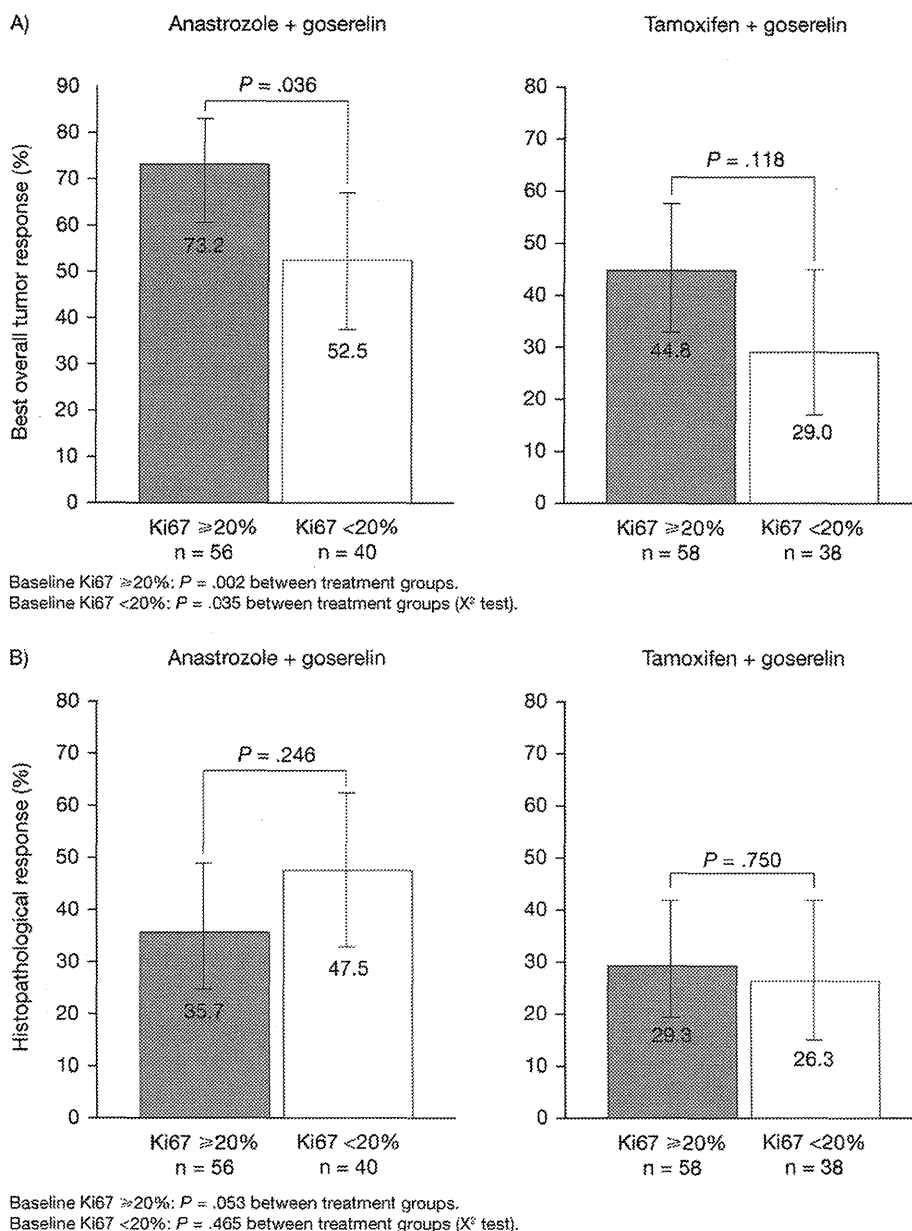


Figure 2. These charts illustrate the baseline Ki-67 index ($\geq 20\%$ vs $< 20\%$) according to (A) the best overall tumor response and (B) the histopathologic response at 24 weeks. Magnetic resonance imaging or computed tomography was used to measure responses. The best tumor response was defined a complete or partial response during the 24-week treatment period.

phase 3 randomized trial that compared tumor response for anastrozole plus goserelin versus response tamoxifen plus goserelin during 24 weeks of neoadjuvant treatment in premenopausal women with ER-positive breast cancer. The primary analysis indicated that the reduction in the Ki-67 index for patients who received goserelin was greater with anastrozole coadministration compared with tamoxifen, suggesting a greater inhibitory effect on tumor

cell proliferation with this treatment combination.²⁰ Given the reported clinical prognostic value of Ki-67 expression after short-term neoadjuvant endocrine therapy for breast cancer,¹⁹ this is in concordance with our finding that anastrozole combined with goserelin demonstrates a superior best overall tumor response compared with tamoxifen plus goserelin. Although Ki-67 is perceived as a reliable predictive endpoint, the outcomes of

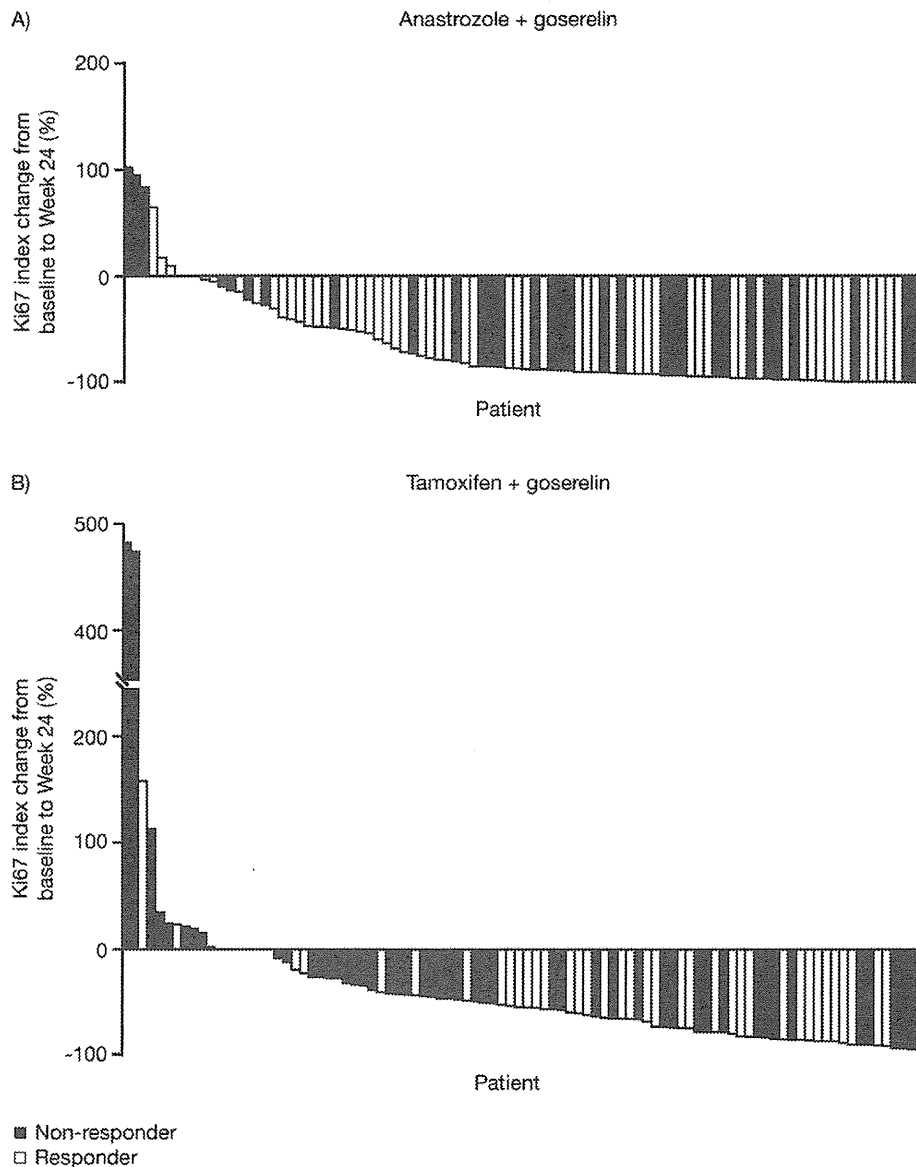


Figure 3. This is a waterfall plot of reductions in nuclear antigen Ki-67 levels in (A) the anastrozole plus goserelin treatment group and (B) the tamoxifen plus goserelin treatment group. Magnetic resonance imaging or computed tomography was used to measure responses. Responders were defined as those patients who had a complete or partial response during the 24-week treatment period.

the parallel adjuvant trial by the Austrian Breast and Colorectal Cancer Study Group (ABCSG) did not reflect outcomes related to the Ki-67 changes we observed: Results from the ABCSG-12 study indicated that there was no difference in disease-free survival between patients who received anastrozole versus tamoxifen (hazard ratio, 1.08; 95% CI, 0.81-1.44; $P = .591$).²⁶ The reason for this difference is not clear, although there were differences in the baseline characteristics of patients in each study: the

STAGE study assessed a more hormone-dependent phenotype of tumor (ER-positive/HER2-negative in the STAGE study vs ER-positive/HER2-negative and ER-positive/HER2-positive in the ABCSG-12 trial), and the proportion of women with a body mass index $>25 \text{ kg/m}^2$ was lower in the STAGE study (17% vs 33%). The ABCSG-12 group did not assess Ki-67 levels. It is also interesting to note that, as recently pointed out by Gonçalves et al,²⁷ in our study, serum estradiol suppression

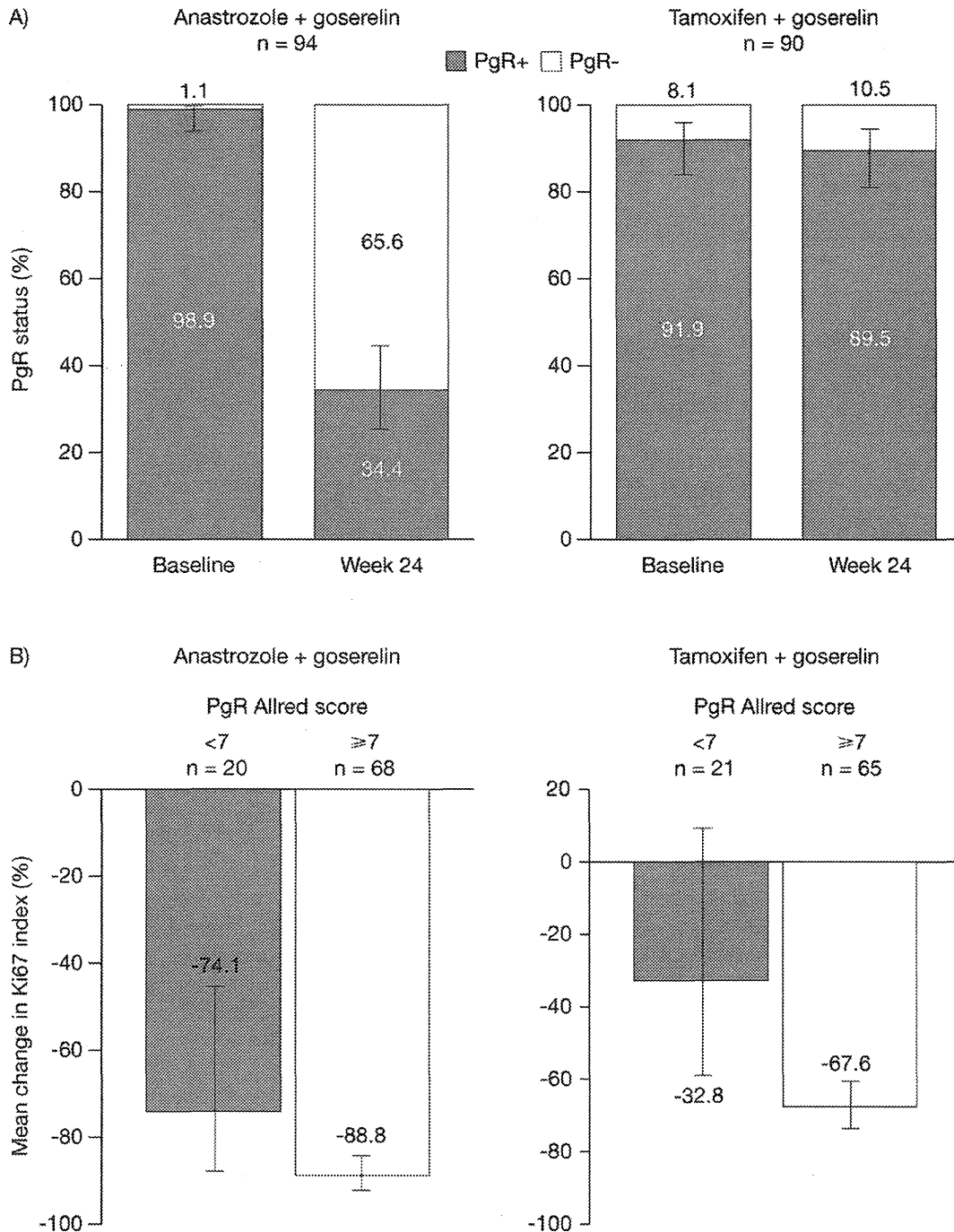


Figure 4. (A) Progesterone receptor status is illustrated at baseline and at 24 weeks. (B) Changes in the Ki-67 index and the baseline PgR Allred score are illustrated. PgR-positive (PgR+) indicates an Allred score >3; PgR-negative (PgR-), an Allred score <2.

appeared to decrease at week 24 compared with week 4, although the suppression was not statistically significant. This suggests the possibility of a gradual tachyphylaxis of the estrogen-suppressing effects of combined goserelin

and anastrozole treatment, which potentially may explain the difference in outcomes between the ABCSG-12 and STAGE studies. However, further investigations would be required to confirm this.

TABLE 2. Preoperative Endocrine Prognostic Index Score

Treatment Group	No. of Patients	PEPI Score: No. of Patients (%)		
		0	1-3	≥4
Anastrozole plus goserelin	84	28 (33.3)	38 (45.2)	18 (21.4)
Tamoxifen plus goserelin	79	9 (11.4)	41 (51.9)	29 (36.7)
<i>P</i> for anastrozole vs tamoxifen		—	—	.002

Abbreviation: PEPI, Preoperative Endocrine Prognostic Index.

^a*P* values were determined using the chi-square test.

In the current study, the best overall tumor response was superior with anastrozole compared with tamoxifen, irrespective of the baseline Ki-67 index. Within the anastrozole treatment group, we observed that the best overall tumor response was significantly better in patients who had a baseline Ki-67 index $\geq 20\%$ versus patients who had a baseline Ki-67 index $< 20\%$. However, in the anastrozole group, we observed a numerically lower histopathologic response in patients who had a baseline Ki-67 index $\geq 20\%$ compared with those who had a baseline Ki-67 index $< 20\%$. It was reported previously that baseline Ki-67 expression was not associated with outcome after neoadjuvant endocrine treatment (including anastrozole, letrozole, and tamoxifen) in ER-positive, postmenopausal women who had breast cancer.^{19,25}

There was no apparent relation between a reduction in the Ki-67 index for responders and nonresponders in either treatment group. Although there tended to be more nonresponders among patients in the tamoxifen group who had less of a reduction in the Ki-67 index, the Spearman rank-correlation between the percentage change in the Ki-67 index and the best percentage change in greatest tumor dimension for the tamoxifen group was a modest 0.314. This observation is essentially consistent with what was reported previously by Dowsett et al, who conducted a similar analysis of postmenopausal patients who received neoadjuvant tamoxifen, anastrozole, and the tamoxifen/anastrozole combination.²⁸ This variation in the Ki-67 index change between responders and nonresponders indicates that the mechanism of estrogen-dependent growth is heterogeneous among breast tumors. Tumor growth is determined by a balance between cell proliferation and apoptosis. Stimulation of cell proliferation by estrogen may be dominantly implicated in tumor growth in some tumors, whereas inhibition of apoptosis by estrogen may be dominantly implicated in other tumors. Thus, a responder does not necessarily have a greater reduction in the Ki-67 index compared with a nonresponder if apoptosis is induced more strongly in the former than the latter after treatment.

In the neoadjuvant setting, endocrine therapy has demonstrated greater (or equivalent) efficacy in postmenopausal women with a lower Ki-67 index.^{29,30} In contrast, in our study, both anastrozole and tamoxifen produced greater response rates in premenopausal women with a higher Ki-67 index. It is therefore possible that the main pathways of proliferative stimulation (and the effectiveness of endocrine treatments) may differ between premenopausal and postmenopausal women with ER-positive breast cancer, according to their level of Ki-67 expression. In general, high Ki-67 expression is traditionally believed to offer a poor prognosis and is predictive of response to chemotherapy regimens.³¹ However, our results suggest that endocrine therapy has at least comparable effectiveness for premenopausal patients with ER-positive breast cancer who have a high Ki-67 index.

No correlation could be determined between a change in the Ki-67 index and baseline ER status in either treatment group. However, the number of patients who were identified as PgR-positive decreased at week 24 in the anastrozole treatment group, an effect that was not observed in the patients who received tamoxifen plus goserelin. PgR expression also was reduced under neoadjuvant AI treatment for breast cancer in the ABCSG 17 study, although it remains to be determined whether the down-regulation of PgR may be used as a marker of clinical efficacy.³² In our study, the reason why the positive rate of PgR was reduced in the anastrozole plus goserelin arm compared with the tamoxifen plus goserelin arm is most likely because of the estrogenic action of tamoxifen, which would induce PgR expression.

Although there may be a potential correlation between a reduction in Ki-67 and the baseline PgR Allred score in patients who receive anastrozole plus goserelin versus tamoxifen plus goserelin, further analyses will be required to determine whether a Ki-67 reduction in patients with high baseline PgR expression translates into a clinical benefit.

After treatment with anastrozole, a lower proportion of patients had a PEPI score ≥ 4 (indicating a high risk of

recurrence) compared with the tamoxifen treatment group. The PEPI model has been validated previously and has indicated significant differences in recurrence-free survival in the adjuvant setting between 3 PEPI risk groups (PEPI risk scores of 0, 1-3, and ≥ 4), with a PEPI score of 0 indicating a very low risk of relapse.²⁵ Data from the adjuvant treatment setting will provide added knowledge for the individualization of future adjuvant treatments after neoadjuvant therapy for breast cancer.

Currently, very little is known about the prognostic effect of Ki-67 in premenopausal women. However, in 1 recent study, the prognostic significance of Ki-67 was investigated in women with ER-positive breast cancer who had received short-term presurgical tamoxifen, and Decensi and colleagues reported that the Ki-67 response was a good predictor of recurrence-free survival and overall survival.³³

To our knowledge, this is the first randomized study to investigate the potential of Ki-67 as a clinical biomarker for AI efficacy in premenopausal women with ER-positive breast cancer. It has been demonstrated that a reduction in Ki-67 expression as a result of neoadjuvant AI treatment can be a potentially useful marker of improved surgical outcomes in postmenopausal women with ER-positive breast cancer, and such a reduction has been identified as predictive of favorable outcomes in the adjuvant treatment period.³⁴ A reduction in Ki-67 expression during neoadjuvant treatment reportedly was greater with anastrozole versus tamoxifen in postmenopausal women who had ER-positive breast cancer,¹⁸ and a parallel result also was observed in the corresponding adjuvant trial, in which recurrence-free survival also was greater for those who received anastrozole.⁸ Yet another similar result was observed for letrozole, in which a greater Ki-67 reduction was observed compared with tamoxifen in the neoadjuvant setting.³⁵ Greater clinical effectiveness also was observed for letrozole in the neoadjuvant setting, both in terms of the objective response rate and the rate of breast-conserving surgery.³⁶

In conclusion, tumor response was greater with anastrozole compared with tamoxifen, regardless of the baseline Ki-67 index, in premenopausal women who received goserelin as neoadjuvant therapy for ER-positive, early stage breast cancer. The current results indicate that endocrine therapy may offer a more tolerable treatment option than cytotoxic chemotherapy as neoadjuvant treatment for these patients, and further studies of the anastrozole plus goserelin treatment combination in this setting are warranted.

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CONFLICT OF INTEREST DISCLOSURES

Dr. Iwase has received honoraria from AstraZeneca and research funding from AstraZeneca; Chugai Pharmaceutical Company, Ltd.; Novartis; and Takeda. Mr. Hayashi is an employee and holds stock ownership with AstraZeneca. Dr. Noguchi has received honoraria and research funding from and has acted in a consultant or in an advisory role for AstraZeneca.

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Locoregional recurrence risk factors in breast cancer patients with positive axillary lymph nodes and the impact of postmastectomy radiotherapy

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Abstract

Background Locoregional recurrence (LRR) after mastectomy reduces the patient's quality of life and survival. There is a consensus that postmastectomy radiotherapy (PMRT) helps establish locoregional control and reduces LRR in patients with ≥ 4 metastatic nodes. However, in patients with 1–3 metastatic nodes, the incidence of LRR and the role of PMRT have been the subject of substantial controversy. This study assessed the risk factors for LRR and the efficacy of PMRT in Japanese breast cancer patients with metastatic nodes.

Methods This study analyzed 789 cases of invasive breast carcinoma with metastatic nodes from 1998 to 2008. We divided the study population into 4 groups: 1–3 positive nodes with/without chemotherapy and ≥ 4 positive nodes with/without chemotherapy. Risk factors for LRR were identified and the relationship between LRR and PMRT was analyzed.

Results During the median follow-up of 59.6 months, 61 (7.7%) patients experienced LRR. In patients who received chemotherapy, independent LRR risk factors were high nuclear grade, severe lymphatic invasion, vascular invasion, and progesterone receptor-negative status in patients with 1–3 positive nodes, and severe lymphatic invasion and estrogen receptor-negative status in patients with ≥ 4 nodes. Although patients treated with PMRT had good outcomes, there was no significant difference, and PMRT did not significantly improve the outcome of the patients with all risk factors.

Conclusions With systemic therapy and adequate dissection, PMRT by itself was of limited value in establishing locoregional control. The indication for PMRT in patients with 1–3 positive nodes remains controversial.

Keywords Breast cancer · Locoregional recurrence · Postmastectomy radiotherapy · Outcome

Introduction

For breast cancer patients and oncologists alike, locoregional recurrence (LRR) is still a clinical problem with regard to control of the disease and outcome after mastectomy. To achieve locoregional control and reduce LRR, the role of postmastectomy radiotherapy (PMRT) has been established by several randomized clinical trials (RCT) [1–4]. Based on these results, PMRT has become the standard adjuvant therapy for patients with 4 or more metastatic lymph nodes. Recently, some RCT demonstrated that PMRT improves outcome in all patients with metastatic lymph nodes, regardless of the number of positive nodes [5, 6]. However, the role of PMRT in patients with 1–3 positive nodes remains controversial [7, 8].

This study is a retrospective analysis to evaluate which clinicopathological features are predictive factors for LRR, such as the number of metastatic nodes. We also analyze the role and efficacy of PMRT in Japanese breast cancer patients.

Materials and methods

Patients and treatments

This study is a retrospective analysis of 789 patients with invasive breast carcinoma with metastatic lymph nodes

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who were treated with total mastectomy and axillary lymph node dissection (level II or level III) at the National Cancer Center Hospital, Tokyo, Japan, from 1998 to 2008.

Neoadjuvant chemotherapy (NAC) was indicated for clinical stage II tumors that were larger than 3 cm in diameter, and for all stage III tumors. Adjuvant chemotherapy and/or hormone therapy were given in cases based on the most current recommendations from the St. Gallen's Consensus Meeting at the time [9–13]. Anthracycline-based chemotherapy included 4 cycles of CEF (cyclophosphamide 500 mg/m², epirubicin 100 mg/m², and fluorouracil 500 mg/m²) every 3 weeks or 4 cycles of AC (doxorubicin 60 mg/m² and cyclophosphamide 600 mg/m²). Taxane chemotherapy included 12 cycles of weekly paclitaxel (wPTX, 80 mg/m²). Concurrent anthracycline and taxane chemotherapy included 4 cycles of AT (doxorubicin 50 mg/m² and docetaxel 60 mg/m²) every 3 weeks. Sequential anthracycline and taxane chemotherapy included 2 cycles of AT followed by 12 cycles of wPTX, AC followed by wPTX, or CEF followed by wPTX. Trastuzumab (first cycle 4 mg/kg, subsequent cycles 2 mg/kg) was added to anthracycline and taxane chemotherapy regimens in patients with overexpression of human epidermal growth factor receptor 2 (HER2).

Radiation therapy (RT) was offered to patients with 4 or more metastatic lymph nodes and/or tumors >5 cm. RT was delivered to the chest wall, including the surgical scar and regional lymph nodes (i.e., supraclavicular, infraclavicular, and axillary), by bilateral X-irradiation using the tangential technique. Because level I and II axillary dissection was performed, RT was performed in the axillary apical area (level III). The parasternal region was not included in the field. The patients were treated using a linear accelerator, and the intended dose was a median absorbed dose in the target volume of 50 Gy, given in 25 fractions over a period of 5 weeks. All patients were simulated with a conventional simulator.

Histopathological analysis

Surgical specimens were examined to determine histological subtype, histological grade (HG), nuclear grade (NG), and the presence or absence of lymphatic or vascular space invasion. Histological subtype was classified using the World Health Organization histological classification of breast tumors [14]. HG was assessed using the Scarff–Bloom–Richardson classification [15]. NG was assessed using the General Rules for Clinical and Pathological Recording of Breast Cancer, 16th Edition [16, 17]. Immunohistochemistry was used to examine tissue samples for the expression of estrogen receptor (ER), progesterone receptor (PgR), and HER2. The cutoff values for ER and

PgR were 10% positive cells. HER2 status was defined based on immunohistochemical staining (IHC). The specimens that were HER2 2+ by IHC were then subjected to fluorescence in-situ hybridization (FISH). HER2-positive samples were defined as those that were HER2 3+ in IHC or HER2 2+ in IHC with an amplification ratio in FISH of ≥ 2.0 . The degree of lymphatic invasion (ly) was classified by hematoxylin and eosin staining as follows: absent, no lymphatic invasion; ly1+, minimal lymphatic invasion; ly2+, moderate lymphatic invasion; and ly3+, marked lymphatic invasion. The degree of venous invasion (v) was classified as follows: absent, no venous invasion; present, venous invasion. These evaluations were performed by two qualified pathologist.

Follow-up and statistical analysis

The duration of follow-up was calculated from the first day of treatment (NAC or surgery) to the most recent medical visit on record. LRR was defined as tumor recurrence in chest wall or regional lymph nodes with or without synchronous distant metastasis.

For comparison of categorical variables, the chi-squared test was used. Locoregional recurrence-free survival (LRFS) was calculated using the Kaplan–Meier method and compared using the log-rank test. Cox's proportional hazards regression models were used to assess the prognostic significance of tumor clinicopathological characteristics on the evaluated outcomes, which were expressed as hazard ratios (HR) with 95% confidence intervals (CI). Factors that were significant at $p \leq 0.05$ in the univariate analysis were entered into the multiple regression models. All data were analyzed using SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Associations between patient and tumor characteristics and LRR

The patients, divided into 2 groups based on the number of positive nodes, and their chemotherapy treatment status are shown in Table 1. All patients had 6 or more dissected lymph nodes; the median number of dissected lymph nodes was 18.6. The median number of positive nodes per patient was 3.0.

In patients with 1–3 positive nodes (n 1–3), the mean age and the proportion of postmenopausal patients were significantly higher in those who did not receive chemotherapy. The patients who received chemotherapy had tumors that were significantly higher in HG and were more likely to be ER-negative. In patients with 4 or more

Table 1 Patient and tumor characteristics

	1–3 positive lymph nodes (<i>n</i> = 479)			≥4 positive lymph nodes (<i>n</i> = 310)		
	Chemotherapy (<i>n</i> = 370)	No chemotherapy (<i>n</i> = 109)	<i>p</i>	Chemotherapy (<i>n</i> = 268)	No chemotherapy (<i>n</i> = 42)	<i>p</i>
Age (years), mean ± SD	52.8 ± 10.5	64.0 ± 12.1	<0.001	54.0 ± 10.6	62.2 ± 13.8	<0.001
Menopausal status			<0.001			0.064
Premenopausal (%)	172 (46.5)	20 (18.3)		105 (39.2)	10 (23.8)	
Postmenopausal (%)	198 (53.5)	89 (81.7)		163 (60.8)	32 (76.2)	
Tumor size (cm), mean ± SD	3.4 ± 2.1	3.2 ± 1.8	0.308	4.8 ± 2.9	4.0 ± 2.0	0.109
Tumor size (mm)			0.297			0.471
<21 (%)	114 (30.8)	33 (30.3)		49 (18.3)	6 (14.3)	
21–50 (%)	196 (53.0)	66 (60.6)		119 (44.4)	23 (54.8)	
>50 (%)	60 (16.2)	10 (9.2)		100 (37.3)	13 (31.0)	
Histological subtype			0.175			0.423
IDC (%)	327 (88.4)	97 (89.0)		237 (88.4)	32 (76.2)	
ILC (%)	24 (6.5)	3 (2.8)		17 (6.3)	2 (4.8)	
Other (%)	19 (5.1)	9 (8.3)		14 (5.2)	4 (9.5)	
Histological grade			0.008			0.598
G1 (%)	19 (5.1)	8 (7.3)		11 (4.1)	3 (7.1)	
G2 (%)	153 (41.4)	62 (56.9)		91 (34.0)	15 (35.7)	
G3 (%)	192 (51.9)	39 (35.8)		160 (59.7)	24 (57.1)	
Nuclear grade			0.052			0.017
G1 (%)	29 (7.8)	13 (11.9)		23 (8.6)	2 (4.8)	
G2 (%)	156 (42.2)	58 (53.2)		80 (29.9)	23 (54.8)	
G3 (%)	176 (47.6)	38 (34.9)		165 (61.5)	17 (40.5)	
Lymphatic invasion			0.954			0.252
Absent (%)	106 (28.6)	33 (30.3)		37 (13.8)	4 (9.5)	
1+ (%)	219 (59.2)	64 (58.7)		116 (43.3)	22 (52.4)	
2+ (%)	43 (11.6)	12 (11.0)		65 (24.3)	15 (35.7)	
3+ (%)	2 (0.5)	1 (0.9)		30 (11.2)	1 (2.4)	
Vascular invasion			0.148			0.254
Absent (%)	340 (91.9)	97 (89.0)		218 (81.3)	38 (90.5)	
Present (%)	30 (8.1)	12 (11.0)		50 (18.7)	4 (9.5)	
No. of dissected lymph nodes, mean ± SD	17.4 ± 5.8	16.0 ± 6.8	0.110	20.7 ± 7.4	20.8 ± 6.8	0.933
No. of positive nodes, mean ± SD	1.8 ± 0.8	1.6 ± 0.9	0.119	10.9 ± 7.9	9.1 ± 6.3	0.186
ER positive (%)	250 (67.6)	95 (87.2)	<0.001	169 (63.1)	32 (76.2)	0.136
PgR positive (%)	247 (66.8)	80 (73.4)	0.204	161 (60.1)	30 (71.4)	0.231
HER2 positive (%)	76 (20.5)	18 (16.5)	0.294	62 (23.1)	8 (19.0)	0.450
Radiotherapy (%)	55 (14.9)	1 (0.9)	<0.001	174 (64.9)	12 (28.6)	<0.001
LRR (%)	18 (4.9)	6 (5.5)	0.109	32 (11.9)	5 (11.9)	0.776

SD standard deviation, IDC invasive ductal carcinoma, ILC invasive lobular carcinoma, G grade, ER estrogen receptor, PgR progesterone receptor, HER2 human epidermal growth factor receptor 2, LRR locoregional recurrence

positive nodes ($n \geq 4$), the mean age was higher in the subgroup who did not receive chemotherapy; however, there was no difference in menopausal status. NG was higher in those who received chemotherapy. There was no difference with regard to hormone receptor status. Nevertheless, the number of metastatic nodes and the use of RT were higher in those who received chemotherapy.

During the median follow-up of 59.6 months, a total of 61 (7.7%) patients suffered LRR. In the 61 cases of LRR, 40 occurred in the skin and/or chest wall and 21 occurred in the regional lymph nodes. The patients were classified into four groups according to the number of lymph node metastases, and chemotherapy. There were 24/479 (5.0%) cases of LRR in the n 1–3 group and 37/310 (11.9%) in the

$n \geq 4$ group. In particular, in the patients who received chemotherapy, the incidence of LRR was 13/370 (3.5%) in the n 1–3 group and 26/268 (9.7%) in the $n \geq 4$ group.

The relationship between clinicopathological characteristics and the incidence of LRR was analyzed (Table 2). In the univariate analysis, NG 3, the severity of lymphatic invasion, the presence of vascular invasion, and hormone receptor-negative status were significant predictors of LRR in the n 1–3 patients who received chemotherapy. In the $n \geq 4$ patients who received chemotherapy, a tumor size >50 mm, the severity of lymphatic invasion, the presence of vascular invasion, and hormone receptor-negative status were significantly associated with LRR. However, in patients who did not receive chemotherapy, there were no factors significantly associated with LRR among the variables tested, regardless of the number of metastatic nodes.

The independent association between tumor characteristics and the risk of LRR, analyzed using Cox's proportional hazards regression models, is shown in Table 3. In the multivariate analysis, among the n 1–3 patients who received chemotherapy, the severity of lymphatic invasion (HR 3.938; 95% CI 1.275–12.163), NG 3 (3.118; 1.001–9.730), the presence of vascular invasion (4.433; 1.384–14.202) and PgR-negative status (0.177; 0.060–0.521) were correlated with worse LRFS. For the $n \geq 4$ patients who received chemotherapy, the severity of lymphatic invasion (HR 4.861; 95% CI 1.896–12.462) and ER-negative status (0.402; 0.161–0.998) were correlated with worse LRFS.

The role of radiotherapy and incidence of LRR

LRR occurred in 40/547 (7.3%) patients who were not treated with RT and 21/242 (8.7%) patients who were treated with RT. There was no significant difference.

Figures 1, 2, and 3 show the Kaplan–Meier curves for outcomes among patients stratified by the number of positive nodes and treatment status. There was no statistically significant difference in the LRFS rate according to RT treatment status, although there was a trend towards better outcomes in the patients who received RT. There were 2/370 (0.5%) and 112/268 (41.8%) patients who received chemotherapy in the n 1–3 and $n \geq 4$ groups, respectively, who had all risk factors for LRR from the multivariate analysis. Figure 4 shows the outcomes among the patients with 4 or more positive nodes who received chemotherapy, considered a high-risk group. There was again a non-significant trend towards better prognosis with RT.

Discussion

Adjuvant therapy has been demonstrated to improve the outcomes of breast cancer patients. In addition to

chemotherapy, PMRT has been shown to significantly reduce the risk of LRR and improve survival from several randomized control trials [1–4]. Following the consensus, we treated patients with massive lymph node metastasis and/or large tumor volume with RT. This report is the retrospective analysis of the role and efficacy of PMRT and the factors associated with LRR in Japanese patients.

To determine the LRR risk factor for each patient's background, we separated patients into four groups according to the number of positive nodes and whether chemotherapy was given. Irrespective of the number of lymph node metastases, the presence of lymphovascular invasion and hormone receptor-negative status were independent risk factors for LRR. The severity of lymphatic invasion was the common factor. NG was an independent factor in patients with 1–3 positive nodes. These variables were also reported in several other studies [18–21]. Therefore, the incidence of LRR was dependent on the malignancy of the tumor and the invasion of the lymphovascular space. The purpose and role of chemotherapy and RT was changed by the patient's status. For the patients with 1–3 metastatic nodes, chemotherapy was performed because of their hormone receptor-negative and/or high-grade tumor basis of the consensus at the time, and the purpose and role of RT were the prevention of chest wall recurrence after the removal of a large tumor rather than regional lymph node recurrence. On the other hand, most of the patients with more than 4 metastatic nodes were eligible for chemotherapy for systemic control of their metastasis and the purpose and role of RT were the control of their lymphovascular invasion.

RCT studies have shown the incidence of LRR to be 8–10% in patients who received chemoradiotherapy and 24–35% in patients who received chemotherapy without RT [1–4]. However, in our institute, the rate of LRR was 8.7% in patients who received RT and 7.3% in those who did not; the significant benefit of RT was not found in all subgroups. Although our patient population was similar to those of other studies, the incidence of LRR was very low in this study, especially in the patients who did not receive RT. Potential reasons for the low incidence of LRR in this study are the differences in the number of dissected lymph nodes and the duration of follow-up. In other studies, level I and/or partial level II lymph node dissection was performed, with the median number of dissected nodes ranging from 7 to 17 [22]. These numbers are lower than the number of dissected nodes at our institute, where level II or III dissection is the standard procedure. Though the role of PMRT for patients with more than 4 metastatic nodes has been established, it cannot be denied that adequate lymph node dissection is essential for locoregional control. Moreover, if it is considered that LRR is one expression of systemic organ metastasis, the role of RT might be limited

Table 2 Hazard ratio of locoregional recurrence-free survival by patient and tumor characteristics at presentation (univariate analysis)

	1–3 positive nodes					
	Chemotherapy (<i>n</i> = 370)			No chemotherapy (<i>n</i> = 109)		
	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
Menopausal status			0.052			0.716
Premenopausal	1			1		
Postmenopausal	3.037	0.990–9.318		0.663	0.073–6.056	
Tumor size (mm)			0.544			0.696
≤50	1			1		
>50	1.472	0.423–5.132		1.044	0.323–290.133	
Histological subtype			0.926			0.672
IDC	1			1		
ILC	0.945	0.291–3.073		0.137	0.001–1346.73	
Histological stage			0.500			0.999
G1/2	1			1		
G3	1.391	0.529–3.655		1.001	0.167–5.996	
Nuclear grade			0.030			0.368
G1/2	1			1		
G3	3.448	1.124–10.575		2.277	0.380–13.645	
Lymphatic invasion			0.046			0.518
Absent/1+	1			1		
2+/3+	2.894	1.019–8.216		2.035	0.342–914.885	
Vascular invasion			0.002			0.583
Absent	1			1		
Present	6.141	1.976–19.092		1.766	0.232–13.547	
Estrogen receptor			0.028			0.270
Negative	1			1		
Positive	0.330	0.123–0.867		0.259	0.023–2.860	
Progesterone receptor			0.004			0.674
Negative	1			1		
Positive	0.239	0.091–0.631		0.597	0.054–6.599	
HER2			0.825			0.540
Negative	1			1		
Positive	0.868	0.247–3.048		0.038	0.021–133.590	
	≥4 positive nodes					
	Chemotherapy (<i>n</i> = 268)			No chemotherapy (<i>n</i> = 42)		
	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
Menopausal status			0.321			0.588
Premenopausal	1			1		
Postmenopausal	0.691	0.333–1.434		0.674	0.322–1.243	
Tumor size (mm)			0.049			0.495
≤50	1			1		
>50	1.544	1.002–3.424		1.875	0.308–11.405	
Histological subtype			0.385			0.574
IDC	1			1		
ILC	1.275	0.648–2.509		4.453	0.879–17.831	
Histological stage			0.094			0.465
G1/2	1			1		
G3	2.070	0.883–4.848		1.969	0.319–12.148	