truly positive (malignant) cases among "suspicious for malignancy" cases.

FNA is a major technique for breast cytodiagnosis. Although other breast cytodiagnosis techniques, e.g., nipple discharge, are likely to involve a slightly higher percentage of inadequate samples, this reporting form would also appear to be applicable to these techniques.

The 6th edition of the General Rules for the Description of Thyroid Cancer (The Japanese Society of Thyroid Surgery) includes a new reporting form for thyroid cytology, which is quite akin to our new reporting form for breast cytology [8]. Although the details of manipulation and the diagnostic criteria differ between these two forms corresponding to the differences in the organs and lesions concerned, the overall design and purpose are the same. We anticipate that this kind of reporting form will be adopted for other organs for which FNA is applicable.

This new reporting form was adopted in the 15th edition of the General Rules for Clinical and Pathological Recording of Breast Cancer [6]. It is, however, likely that this new form will need revision in the future. We hope that modification of this form, if it requires revision in the future, will be evidence-based.

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Breast Cancer Res Treat (2008) 110:531–539
DOI 10.1007/s10549-007-9744-z

CLINICAL TRIAL

Phase II study of preoperative sequential FEC and docetaxel predicts of pathological response and disease free survival

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Received: 23 August 2007 / Accepted: 23 August 2007 / Published online: 19 September 2007

Abstract *Purpose* This multicenter phase II study examined the impact of pathological effect on survival after preoperative chemotherapy in Japanese women with early stage breast cancer. *Patients and methods* Prior to surgery, patients received four cycles of FEC (fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2, cyclophosphamide 500 mg/m^2 q3w) followed by four cycles of docetaxel (75 mg/m^2 q3w). Primary endpoint was 3 year disease free survival (DFS) stratified by the absence or presence of Quasi-pCR (QpCR; absence of invasive tumor or only focal residual tumor cells). Secondary endpoints were predictors for QpCR, clinical response, breast conservation rate, and safety. *Results* Between June 2002 and June 2004, 202 women were enrolled. Among 191 assessable patients, 25% achieved QpCR. With 40 months median follow-up, 3 year DFS was estimated at 91% for all patients. 3 year DFS for patients with QpCR was 98% vs. 89% without QpCR (hazard ratio 0.38 [95% Confidence Interval 0.09–0.84], $P = 0.0134$). HER2 status and response to FEC were independent predictors of QpCR. The overall clinical

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response was 75%; 85% of patients achieved breast conservation. Grade 3/4 neutropenia was the most common adverse event, observed in 44% and 35% of patients during FEC and docetaxel, respectively. Treatment related side effects were manageable; there were no treatment related fatalities. *Conclusion* FEC followed by docetaxel is an active and manageable preoperative regimen for women with early stage breast cancer. QpCR following preoperative chemotherapy predicts favorable DFS. HER2 overexpression and clinical response to FEC predict QpCR.

Keywords Clinical trial · Docetaxel · Early stage breast cancer · FEC · Preoperative chemotherapy · Phase II

Introduction

Preoperative systemic chemotherapy has been widely used for patients with operable breast cancer to increase the chance for breast conservation [1–3]. Furthermore, response to preoperative treatment can provide information on long-term survival outcomes. Pathological complete response (pCR) in the breast and axillary lymph nodes predicts a favorable prognosis, whereas non-pCR of the breast or node-positive status does not, which can facilitate tailoring of subsequent treatment [1, 3]. In addition, correlative studies of tumor samples before and after treatment may provide information on markers that could predict response or resistance to treatment [4].

Results from the National Surgical Adjuvant Breast and Bowel Project (NSABP) study B-18 demonstrated the impact of preoperative chemotherapy in patients with operable early stage breast cancer [5]. The protocol-specified anthracycline-containing regimen of four cycles of doxorubicin and cyclophosphamide (AC), resulted in an increased chance of breast-conserving surgery (BCS) compared to no preoperative chemotherapy. The study established pCR as a prognostic marker for long-term disease-free survival and demonstrated that there was no difference in survival whether chemotherapy was administered before or after surgery. Subsequently, studies such as the Aberdeen trial have demonstrated the benefit of the sequential addition of taxanes to preoperative anthracycline regimens [6, 7]. NSABP Protocol B-27 demonstrated that compared to preoperative AC alone, the addition of sequential docetaxel doubled the pCR rate, increased the clinical complete response (cCR) rate, and increased the proportion of patients with negative axillary nodes [3, 7]. Although NSABP B-27 did not show that the addition of docetaxel to AC significantly improved disease free survival (DFS) and overall survival (OS) compared to AC alone, other studies, mainly of patients with node-positive disease, have shown favorable DFS and OS by including a taxane with an anthracycline, either in sequence or combination [8–12]. Multiple neoadjuvant studies demonstrated that patients with pathological complete response to chemotherapy had a good prognosis [1, 2].

Here we conducted a multicenter prospective neoadjuvant trial with four cycles of fluorouracil, epirubicin, and cyclophosphamide (FEC) followed by four cycles of docetaxel in Japanese patients with operable breast cancer to investigate the relationship between pathological effect and survival. The pathological effect was determined using the definitions of Quasi-pCR (QpCR: complete disappearance of invasive carcinoma in the breast or only focal tumor cells remaining in the stroma in the removed breast) [13]. The primary endpoint was to examine 3 year DFS stratified by pathological response (QpCR versus non-QpCR). We also performed a logistic regression analysis to examine which features were associated with QpCR with this regimen. Clinical response, the rate of BCS, and safety were also evaluated.

Methods

Study design and ethics

This multicenter, open-label, single-arm, phase II clinical study was conducted at 13 institutions throughout Japan. This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The protocol was reviewed and approved by the institutional review board of each participating institution and written informed consent was obtained from all patients prior to the study.

Patients

Women aged 20–59 years of age with histologically proven early stage breast cancer (T1c-3 N0 M0/T1-3 N1 M0)

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were enrolled. No prior chemotherapy, radiotherapy, hormonal therapy, or immunotherapy was allowed. Other inclusion criteria were the following: Eastern Cooperative Oncology Group performance status of 0–1; white blood cell count between 4000/mm^3 and 12000/mm^3; neutrophil count ≥2000/mm^3; platelet count ≥100000/mm^3; hemoglobin ≥9.5 g/dl; serum bilirubin <1.25 times upper normal limit (UNL), creatinine <1.5 times UNL, or AST and ALT <1.5 times UNL. Patients with congestive heart failure or left ventricular ejection fraction ≤60% were excluded. Patients were also excluded if they had confirmed infection; serious concomitant illness such as severe cardiovascular disease, uncontrolled diabetes, malignant hypertension and hemorrhagic disease; active concomitant malignancy; brain metastasis; interstitial pneumonia or lung fibrosis confirmed by chest X-ray or computed tomography; pleural or peritoneal effusion that required treatment; pericardial effusion; motor paralysis, peripheral neuropathy or edema history of severe drug allergy; or had previously received long-term corticosteroid therapy. Pregnant or lactating women were also excluded.

Treatment procedures

Four cycles of FEC (fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2, and cyclophosphamide 500 mg/m^2) administered intravenously (i.v.) on day 1 every 21 days were followed by four cycles of docetaxel i.v. (75 mg/m^2) every 21 days, prior to surgery. The doses of docetaxel and epirubicin selected at the time of this study were higher than the approved doses in Japan (60 mg/m^2 each). Premedication consisted of a 5-HT$_3$ antagonist and dexamethasone i.v. on day 1 with oral dexamethasone on days 2 and 3 with each cycle of FEC and dexamethasone i.v. with or without 5-HT$_3$ antagonist on day 1 with each cycle of docetaxel. Administration of recombinant human granulocyte colony-stimulating factor (rh G-CSF) and antibiotics was left to the judgment of each investigator. If patients prematurely discontinued FEC treatment, they were expected to proceed to four cycles of docetaxel.

Treatment could be postponed for a maximum of 2 weeks for severe toxicity. If toxicity did not improve during this period, chemotherapy was discontinued and surgery was recommended. Dose reductions of epirubicin from 100 mg/m^2 to 75 mg/m^2 and for docetaxel from 75 mg/m^2 to 60 mg/m^2 were permitted in case of febrile neutropenia and grade 3 or 4 non-hematological toxicities except for nausea, vomiting, and fatigue. Following chemotherapy and clinical assessment of response, patients underwent surgery. If the tumor was too large or invasive for breast-conserving surgery, modified radical mastectomy was recommended. Sentinel lymph node biopsy (SNB) was performed to confirm disease stage. Most patients with negative biopsies did not undergo surgical clearance of axillary nodes. Autologous or heterologous reconstructive surgery was performed as needed. All patients who underwent breast-conserving surgery were given standard radiotherapy to the remaining ipsilateral breast tissue after surgical recovery. For patients with node-negative status in the sentinel nodes not requiring axillary dissection, radiotherapy to the axilla was allowed but not required. No recommendations were made for post-study hormone therapy in the protocol.

Assessment

Hormone receptor and HER2 overexpression

Estrogen receptor (ER) status and progesterone receptor (PgR) status were determined by immunohistochemistry at each institute. In general, tumors with >10% positively stained tumor cells were classified positive for ER and PgR. HER2 status was also determined at each institute by immunohistochemistry or by fluorescence in situ hybridization (FISH) analysis. HER2 positive tumors were defined as 3+ on immunohistochemistry staining or as positive by FISH.

Central pathological assessment

Haematoxylin and eosin (H&E) and keratin stained slides were prepared as 5 mm tissue sections from the primary tumor. Pathological breast tumor response was assessed by a central review committee consisting of three pathologists using modified criteria of the Japanese Breast Cancer Society [14]. A blinded central review committee evaluated the pathologic response independently to the local pathologists. In this study, the response of stromal invasion and intraductal component was assessed separately. Cytokeratin immunostaining was performed to confirm residual cancer cells in required cases.

Toxicity and clinical assessment

Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria (version 2). Tumor response was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines in patients who had measurable lesions. Tumor and toxicity assessments were performed within 4 weeks prior to FEC treatment, after completion of FEC treatment, and before surgery.

Statistical methods

The primary endpoint was to examine 3 year DFS stratified by pathological response (QpCR versus non-QpCR). Secondary endpoints included predictors for QpCR, clinical response, the rate of BCS, and safety.

For the primary efficacy analysis, we assumed that approximately 25% of patients would achieve QpCR and that the 3 year DFS rate in patients with non-QpCR would be 70%. To demonstrate a 20–25% reduction in the hazard of DFS between patients achieving QpCR compared with those without QpCR, we planned to enroll 200 patients. Using the log rank test this would provide $\alpha = 0.05$ and $\beta = 0.2$.

Kaplan–Meier analysis was used to estimate the values of DFS. DFS was compared using a log-rank test stratified for QpCR and non-QpCR. Events for the calculation of DFS include all local, regional, or distant recurrence, all clinically inoperable and residual disease at surgery, all second cancers, contralateral breast cancers, and all deaths.

In the logistic regression analyses, adjustments were made for the stratification variables of menopausal status, tumor size, estrogen receptor status, progesterone receptor status, HER2 status, clinical response to FEC treatment and clinical response to docetaxel following FEC treatment. Analyses were performed with JMP (version 6, SAS Institute Inc.). Analyses of endpoint data reported here are based on information received as of July 2007.

Results

Patient characteristics

Between June 2002 and June 2004, 202 patients were prospectively enrolled. As two patients were ineligible and two patients withdrew consent, 198 patients were assessed for safety. One patient was removed from the study after planned chemotherapy but before surgery because of a protocol violation (non-protocol chemotherapy), four patients elected to not have surgery and withdrew from the study, and two were lost to follow-up, leaving 191 evaluable for clinical, pathologic assessment and DFS.

The median age of the assessable 198 patients was 46 years, and 72% of patients were pre-menopausal. The majority of the patients had T2 tumors (74%), with 20% of the patients having T3 tumors and 6% with T1 tumors (Table 1). Distribution with regard to hormone receptor or HER2 overexpression was representative of that seen in common practice in Japan [15].

Table 1 Patients characteristics (n = 198)

	No. of patients	%
Age (years)		
Median	46	
Range	25–60	
Menopausal status		
Pre	142	72
Post	56	28
Tumor stage		
T1	12	6
T2	146	74
T3	40	20
Nodal stage		
N0	80	40
N1	117	59
N2	1	1
Hormone receptor status		
ER		
Positive	133	67
Negative	62	31
Unknown	3	2
PgR		
Positive	100	51
Negative	95	48
Unknown	3	2
HER2 (IHC)		
0	60	30
1+	54	27
2+	42	21
3+	38	19
Unknown	4	2

ER estrogen receptor, *PgR* progesterone receptor, *IHC* immunohistochemistry

Percentages may not add up to 100% because of rounding

Compliance to chemotherapy and toxicity

Dose reduction due to toxicities was made in 18% of the patients during FEC treatment; febrile neutropenia (19), grade 3–4 neutropenia without fever (10), suspicion of febrile neutropenia (4), vomiting, and deterioration in liver function (1 each) and 14% of patients during docetaxel therapy, febrile neutropenia (5), grade 3–4 neutropenia without fever (5), neutropathy (2), deterioration in liver function (2), myalgia (2) allergy (1) previous reduction of FEC (8), and unknown (2).

Six patients (3%) discontinued FEC treatment due to toxicities (3: two patients with febrile neutropenia and one with vomiting), progression of disease (2), and mental disorder (1). Ten (please refer toxicity section) patients (5%) discontinued docetaxel treatment due to toxicity (3:

one patient each with rash, febrile neutropenia, and phototoxicity), progression of disease (3), and patients' requests for early surgery (2) changing hospital (1), patient's request (1).

Percentage of treatment cycles requiring dose reduction for FEC, docetaxel and all were 11.1, 11.6 and 11.3%. Percentage of treatment cycles (FEC, docetaxel and all) including rh G-CSF were 10.5, 8.2 and 9.4%, respectively.

The safety profile is summarized in Table 2. Four patients didn't receive docetaxel treatment at patients' request. For toxicity 198 and 194 patients were evaluable for FEC treatment and docetaxel treatment, respectively. The most common adverse event was grade 3 or 4 neutropenia, which was observed in 44% of patients during FEC treatment and 35% of patients during docetaxel treatment. Fever, including febrile neutropenia, was seen in 20% and 7% during treatment with FEC and docetaxel, respectively. The only grade 3–4 non-hematologic toxicities reported were; nausea (12 patients), vomiting (11) and fatigue (3). No fatal events were observed.

Response to treatment

The overall clinical response was 74% (95% CI, 67–80%) with 22% CR and 52% PR. Thirty-eight (51%) of 75 FEC non-responders had a response to docetaxel treatment. One hundred and six of 118 FEC responders maintained their response or had a continued decrease in tumor size with docetaxel (Table 3). QpCR were seen in 25% of patients (including 16% complete disappearance of invasive carcinoma in the breast). One patient was removed from assessable for BCS because of a protocol violation. BCS was achieved in 85% of all the assessable patients. Ninety-two percent of patients who had original tumor size 3 cm or less underwent BCS; those with larger tumors had an 80% rate of BCS. As of July 11, 2007, with a median follow up of 40 months, the estimated 3-year DFS was 91% for all patients. Patients who achieved QpCR had significantly improved DFS compared to those without QpCR (QpCR (98%) and non-QpCR (89%), log rank test, $P = 0.0333$, Fig. 1). HR 0.38 [95% CI 0.09–0.84], $P = 0.0134$).

Predictive factors of pathological response

A multiple logistic regression analysis was performed to examine which factors among menopausal status, tumor size, estrogen receptor status, progesterone receptor status, HER2 status and clinical response to FEC were associated with QpCR (Table 4). HER2 status and response to the initial FEC treatment and response to docetaxel were independent predictive factors for QpCR. The QpCR rates stratified by HER2 and ER are shown in Fig. 2. QpCR rate was 67, 33, 35 and 13% in HER2 positive/ER negative, HER2 positive/ER positive, HER2 negative/ER negative, HER2 negative/ER positive, respectively.

Table 2 Treatment related toxicities

	FEC (n = 198)		Docetaxel (n = 194)	
	All grades n (%)	Grade 3, 4 n (%)	All grades n (%)	Grade3, 4 n (%)
Non-hematologic toxicities				
Fatigue	83 (42%)	2 (1%)	83 (42%)	1 (1%)
Diarrhea	17 (9%)	1 (1%)	31 (16%)	0
Nausea	162 (82%)	11 (6%)	81 (42%)	1 (1%)
Vomiting	98 (50%)	10 (5%)	38 (20%)	1 (1%)
Neurotoxicity	6 (3%)	0	85 (44%)	2 (1%)
Constipation	67 (34%)	0	50 (26%)	1 (1%)
Arthralgia/myalgia	12 (6%)	0	60 (30%)	1 (1%)
Hematologic toxicities				
Hemoglobin	119 (60%)	1 (1%)	101 (52%)	0
Platelets	26 (13%)	1 (1%)	3 (2%)	1 (1%)
AST/ALT	81 (41%)	3 (2%)	70 (36%)	1 (1%)
Leukocytes	131 (66%)	68 (35%)	92 (47%)	57 (30%)
Neutrophils	137 (69%)	85 (44%)	85 (44%)	67 (35%)
Febrile neutropenia	–	40 (20%)	–	14 (7%)

FEC fluorouracil, epirubicin, cyclophosphamide

Table 3 Clinical response after FEC and after docetaxel following FEC treatment (n = 194)

Clinical response, *N* (%)	Overall	
	Responder	Non-responder
FEC		
Responder	106 (90%)	13 (10%)
Non-responder	38 (51%)	37 (49%)

cCR + *cPR* responder, *cSD* + *cPD* non-responder, *FEC* fluorouracil, epirubicin, cyclophosphamide, *CI* confidence interval

Discussion

We have presented results from the largest study to date that enrolled Japanese women undergoing preoperative chemotherapy for early stage breast cancer. Our findings demonstrated that four cycles of preoperative FEC followed by four cycles of docetaxel conferred a high rate of BCS, even among patients with primary tumors larger than 3 cm. We found a significant improvement in DFS when QpCR could be achieved, compared to the absence of QpCR. HER2 overexpression, response to FEC and response to docetaxel were significant predictors of QpCR with this regimen.

Regarding toxicity, there were no fatal events and no significant differences in the types and severity of toxicity as compared to other recent studies using similar regimens outside of Japan [6, 8, 9, 16–18]. Compared with overseas studies that also did not allow rh G-CSF the incidence of fever was the same in this study [8, 19]. In another studies which showed lower incidence of febrile neutropenia (13.5%) all patients were treated with rh G-CSF [16].

One of the merits of neoadjuvant chemotherapy for operable breast cancer is to decrease the size of the primary tumor in order to allow for BCS. The study protocol did not provide guidelines for breast conservation; therefore, the BCS rate that we observed reflected the biases that may occur in real-life clinical practice in Japan. Nevertheless, the BCS rate of 80% that we observed was favorable compared with other neoadjuvant studies performed overseas [3, 16].

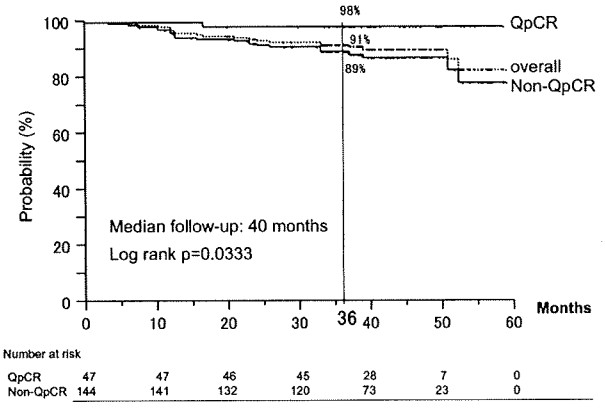

Fig. 1 Relationship of QpCR and non-QpCR to disease free survival

The PACS 01 trial which compared six cycles of adjuvant FEC with a sequential regimen of three cycles of FEC followed by three cycles of docetaxel 100 mg/m^2 (FEC-D) demonstrated an 18% risk reduction in DFS and 27% risk reduction in OS with FEC-D (adjusted P = 0.017). This study supports the conclusions that sequential adjuvant chemotherapy with FEC followed by docetaxel significantly improves DFS and OS in node-positive breast cancer patients [9]. In the current study the dose of docetaxel 75 mg/m^2 was selected based on the recommended doses for docetaxel in Japan, and we showed that the actual 3-year DFS rate of 91% was better than expected based on the results of overseas studies [7, 9, 20]. This confirms that the approved doses of 75 mg/m^2 is an appropriate does in Japanese women.

Furthermore a new definition of QpCR was defined for pathological effect in this study. When stratified between QpCR and non-QpCR, patients with QpCR had significantly favorable DFS. Indeed by adding docetaxel to FEC patients with QpCR resulted in improved survival similar to previous studies.

Even without anti-HER2 targeting therapy, a QpCR rate >60% was achievable in ER negative and HER2 positive tumors. A multivariate analysis has indicated the significant value of HER2 overexpression, which seems to suggest the importance of HER2 in the prediction of QpCR with this regimen. In this study both an anthracycline and docetaxel were used, so it is not clear which treatment was more strongly associated with HER2 as a predictive value of QpCR. Data in the metastatic and adjuvants setting suggest that docetaxel regimens may be more active than non docetaxel regimens in HER2 positive tumors [8, 21]. The value of HER2 status as a predictor of response to anthracycline-based chemotherapy is still a matter debate. On the other hand, there are several implicative data showing the predictive value of topoisomerase (Topo)-II for anthracyclines because Topo-II is a molecular target of anthracyclines [22–25]. There is evidence that HER2 amplification and Topo-II amplification usually occur in parallel and it is rare to have Topo-II amplification without HER2 amplification [23, 26]. In this study QpCR rate might clarify the difference between HER2 positive tumors and HER2 negative tumors. No patient has received trastuzumab in the adjuvant setting. Future translational studies will be necessary to explore the significance of Topo-II amplifications as well as HER2 gene amplifications in the prediction of the pathological response of this regimen. This result will be included the information in the future if

Table 4 Predictive variables for QpCR

Variables	Before treatment OR 95% CI (*P*)	After FEC treatment OR 95% CI (*P*)	After docetaxel following FEC treatment OR 95% CI (*P*)
Menopausal status	1.43	1.38	1.37
Pre (versus post)	0.94–2.15 (NS)	0.89–2.14 (NS)	0.87–2.12 (NS)
Tumor size	0.89	0.93	0.87
>3 cm (vs ≤3 cm)	0.61–1.3 (NS)	0.63–1.37 (NS)	0.59–1.28 (NS)
ER	1.4	1.44	1.35
Negative (versus Positive)	0.87–2.27 (NS)	0.88–2.36 (NS)	0.81–2.23 (NS)
PgR	1.61	1.49	1.65
Negative (versus Positive)	0.97–2.67 (NS)	0.89–2.51 (NS)	0.98–2.79 (NS)
HER2	2.02	2.24	2.11
3+ (vs <3+)	1.31–3.11 (0.0014)	1.42–3.53 (0.0005)	1.36–3.3 (0.0009)
Clinical response to FEC treatment	–	1.78	–
Response (versus non-response)	–	1.15–2.76 (0.0096)	–
Clinical response to docetaxel following FEC treatment	–	–	1.99
Response (versus non-response)	–	–	1.14–3.47 (0.0154)

QpCR quasi pathological complete response, *FEC* fluorouracil, epirubicin, cyclophosphamide, *OR* odds ratio, *ER* estrogen receptor, *PgR* progesterone receptor, *CI* confidence interval, *NS* not significant

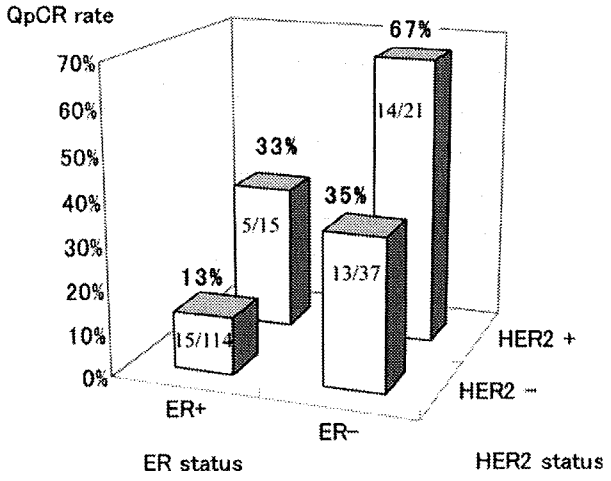

Fig. 2 Relationship between QpCR and HER2/ER status (n=187)

we use anthracycline and trastuzumab for all HER2 positive patients.

In the present study, though a multivariate analysis hasn't indicated the significant value of the status of hormone receptor, QpCR rate was higher in ER negative tumors than ER positive tumors, and QpCR rate in ER negative and HER2 positive tumors was remarkably high compared with ER positive and HER2 negative tumors. This model suggests that ER status is a dependent predictor, for QpCR possibly because it is related to HER2 expression. The sample size was perhaps too small to effectively determine the true impact of ER negative status as a predictor of QpCR. As most patients who are HER2 positive are also ER negative, it is likely that ER status will have some predictive value. However, larger studies are needed to determine this. These results are important for considering individual preoperative systemic therapy. This trend was similar to previous studies using AC followed by paclitaxel regimens, though the therapeutic situations are different [10, 12, 27, 28]. According to recent meta-analyses of post-operative adjuvant therapy, chemotherapy including cyclophosphamide/methotrexate/5FU (CMF)-type regimens, anthracycline-containing regimens and anthracycline followed by paclitaxel are more effective for hormone receptor negative tumors than for hormone receptor positive tumors [10–12, 27–32]. However, while hormone receptor negative tumors may be more responsive to preoperative regimens, a survival benefit can be observed regardless of receptor status [2]. In this study a multivariate analysis hasn't indicated the significant value of the status of hormone receptor. This may be affected by addition of docetaxel. Dose response with anthracycline is also different between hormone receptor positive tumors and hormone receptor negative tumors. For ER negative tumors, higher anthracycline doses may be required for improved prognosis, however, for ER positive tumors it might not be necessary [29].

In this study, most tumors responded to docetaxel even if they did not respond to FEC. However, some tumors showed a response to the initial therapy but a lesser response to the second therapy. This underscores the need to include non-cross resistant treatments in the

management of early stage breast cancer [33]. Various non-cross resistance molecules may be involved in this clinical phenomenon. Recent investigations indicate that initial chemotherapy may change the phenotype of the tumor by inducing pro-survival molecules in tumor cells or stroma [2, 3, 5, 7, 16]. In particular, key mediators such as nuclear factor-kappa B, cyclooxygenase-2 and thymidine phosphorylase are known to be induced by chemotherapy frequently, which may change those tumors relatively anti-apoptotic to the second chemotherapy [34–36]. From the clinical point of view, it would be useful to modify the treatment schedule based on initial response to treatment. Since the types of pro-tumor molecules and the magnitude of induction are different between agents, it might be reasonable to consider a different sequence (taxane followed by anthracycline), if information on the tumor phenotype could be obtained before starting treatment. Various treatment scenarios for non-responders to FEC could be considered. According to recent study results, surgery might be an option for non-responders to initial anthracyclines [37]. In order to enhance the effect of docetaxel, the combination with fluoropyrimidines such as capecitabine may be an option. Obviously for HER2 overexpressing tumors, anti-HER2 containing therapy should be considered. For the ER positive and HER2 negative phenotype, hormone therapy might be an option if tumors are relatively well differentiated. Individual treatment based on ER/HER2 status and the clinical response to the initial anthracyclines may be integrated as future direction [37].

In conclusion, 8-cycle preoperative chemotherapy with non-cross resistant regimens, FEC followed by docetaxel, is safe, feasible, and effective as primary systemic therapy for women with early stage breast cancer. In particular, the regimen allows a majority of Japanese patients to avoid the need for mastectomy. Patients with QpCR demonstrated significantly superior survival results. HER2 over-expression, response to FEC and response to docetaxel were significant predictors for QpCR. Based on our results, preoperative FEC followed by docetaxel should be considered a standard option for the treatment of Japanese women with operable breast cancer.

Acknowledgments We wish to thank the patients who participated in the JBCRG 01 clinical trial. We also thank Drs. Shunichi Inamoto, Tadashi Kobayashi, and Taketo Mukaiyama for their helpful advice as the members of efficacy and safety evaluating committee. We are also grateful to sanofi-aventis, Dr. Steven Olsen, Dr. Takashi Shimamoto, Arika Yoshida, Tomoko Kawamoto, Takeshi Mera and Daisuke Nozaki, for their scientific advice. This study was sponsored by Osaka Cancer Research Foundation and Advanced Clinical Research Organization.

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J Cancer Res Clin Oncol (2008) 134:715–722
DOI 10.1007/s00432-007-0343-5

ORIGINAL PAPER

Histopathological assessment of anastrozole and tamoxifen as preoperative (neoadjuvant) treatment in postmenopausal Japanese women with hormone receptor-positive breast cancer in the PROACT trial

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Received: 21 September 2007 / Accepted: 29 November 2007 / Published online: 13 December 2007

Abstract
Purpose The PReOperative 'Arimidex'® Compared with Tamoxifen (PROACT) trial compared neoadjuvant anastrozole and tamoxifen in postmenopausal women with large, operable or potentially operable, locally advanced hormone receptor-positive breast cancer. Here, we compare objective clinical responses with histopathological tumor responses to therapy in a cohort of 97 Japanese patients, in order to investigate the consistency of assessment methods and the change in estrogen-receptor (ER) and progesterone-receptor (PgR) status.
Methods Histopathological response and the change in ER and PgR status were assessed by comparing pathological specimens collected at baseline (via needle biopsy) with those collected at 3 months (from excised tumors). The response was evaluated using Pathological Response Criteria for Breast Cancer as defined by the Japanese Breast Cancer Society. The patients were randomized to receive anastrozole ($n = 48$) or tamoxifen ($n = 49$).
Results A numerically greater histopathological response rate was observed when neoadjuvant anastrozole compared with neoadjuvant tamoxifen (35.4 and 12.2%, respectively). The histopathological and clinical objective response rates agreed in 63/97 patients. The ER status of 5/40 patients changed from positive at baseline to negative at 3 months in the anastrozole group compared with 20/37 patients in the tamoxifen group. The PgR status of 16/17 patients in the anastrozole group and of 1/11 patients in the tamoxifen group changed from positive to negative.
Conclusions These data support the findings of the main PROACT trial, which confirmed that anastrozole, as compared with tamoxifen, is an effective neoadjuvant endocrine treatment in objective response rates for postmenopausal women with large operable hormone-receptor positive breast cancer. Further follow-up is required to confirm whether histopathological responses to therapy correlate with an overall improvement in survival.

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Keywords Anastrozole · Breast neoplasms · Histology · Neoadjuvant therapy · Pathology · Postmenopause · Tamoxifen

Introduction

The concept of preoperative or neoadjuvant therapy to achieve tumor downstaging prior to surgery is not new. Downstaging may be clinically significant, in that a tumor considered inoperable may become operable, or more conservative surgery may become feasible, where previously mastectomy was the only possible option. Neoadjuvant cytotoxic chemotherapy has already proven effective in downstaging tumors and in increasing the number of operable cancers in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-18 trial (Wolmark et al. 2001). Analysis of data from the NSABP B-18 trial ($n = 1,523$) confirmed that clinical response to neoadjuvant chemotherapy was significantly correlated with treatment outcome for disease-free survival ($P = 0.0008$), recurrence-free survival ($P = 0.0002$), and overall survival ($P = 0.05$) at 9 years follow-up. Overall survival at 9 years was 78% in patients achieving a clinical complete response (cCR; i.e. the absence of clinical evidence of breast tumor on physical examination). However, pathological response differentiated the patients achieving a cCR in terms of outcome. Patients with a pathological complete response (pCR; i.e. no histological evidence of invasive carcinoma on pathological examination of the surgical specimen) showed improved overall survival at 9 years compared with patients achieving a cCR who had residual invasive cancer on pathological examination (overall survival 85 and 73%, respectively) (Wolmark et al. 2001). Patients with a pCR had a reduction of 50% in risk of death compared with the group as a whole [relative risk 0.50; 95% confidence intervals (CI) 0.32, 0.78]. These data, therefore, suggest that pathological response to neoadjuvant treatment is a significant prognostic factor for survival (Kurosumi 2004).

More recent data indicate an association between the expression of estrogen and progesterone receptors (ER and PgR, respectively) and therapeutic response in terms of event-free survival (Vyzula et al. 2004). Patients with tumors expressing both ER and PgR experienced significantly better event-free survival than those with tumors with other hormone-receptor profiles. It is, therefore, logical to investigate endocrine therapies for neoadjuvant treatment, as they might be expected to be effective in downstaging hormone receptor-positive tumors.

Studies reporting the benefits of neoadjuvant tamoxifen, and more recently aromatase inhibitor treatment, have been published (Dixon et al. 2000; Eiermann et al. 2001; Ellis et al. 2001; Mansi et al. 1989; Preece et al. 1982; Semiglazov et al. 2003), although the effect (including the pathological effect) of neoadjuvant treatment on response to consequential adjuvant treatment has yet to be studied.

In the 'Arimidex', Tamoxifen, Alone or in Combination (ATAC) trial, anastrozole proved to be more effective (in terms of disease-free survival) and better-tolerated than tamoxifen as adjuvant therapy for postmenopausal women with hormone receptor-positive breast cancer (Trialists' Group 2005). It is, therefore, reasonable to compare their relative efficacies in the neoadjuvant setting and, furthermore, non-comparative studies have already reported beneficial outcomes for neoadjuvant treatment with anastrozole (Dixon et al. 2000; Anderson et al. 2002).

The PReOperative 'Arimidex'® Compared with Tamoxifen (PROACT; NCT00232661) trial confirmed that is compared with tamoxifen, anastrozole is an effective preoperative treatment for postmenopausal women with large, operable or potentially operable (T2-4b, N0-2, M0) hormone receptor-positive breast cancer (Cataliotti et al. 2006). Here, we look beyond objective-response data evaluating the histopathological response and the change in ER and PgR status at the end of the preoperative period in the Japanese cohort of the PROACT study. Some of the results were reported by Tsuda et al. (2005) at the American Society of Clinical Oncology annual meeting in 2005.

Patients and methods

Patients

Postmenopausal women with large, operable or potentially operable, locally advanced, ER-positive and/or PgR-positive breast cancer were recruited from 25 study centers in Japan over a period of 25 months.

Eligible patients had to satisfy the following criteria:

1. Have operable (T2 [≥3 cm], T3, N0-2, M0) or potentially operable, locally advanced (T4b, N0-2, M0), measurable breast cancer (≥3 cm in diameter, measured using both ultrasound and caliper methods)
2. Have histologically and cytologically proven ER-positive and/or PgR-positive invasive breast cancer using needle-biopsy specimens (Kurosumi 2003).
3. Be likely to benefit from neoadjuvant endocrine therapy, in the opinion of the investigator. (Patients were not included if the investigator judged that other therapy, e.g. chemotherapy should be administered.)
4. Be considered postmenopausal (defined as patients aged ≥60 years, or aged 45–59 years with amenorrhea for ≥12 months and an intact uterus, or with amenorrhea

for <12 months and postmenopausal levels of follicle-stimulating hormone [including patients who had undergone a hysterectomy or were receiving hormone-replacement therapy], or patients who had undergone bilateral oophorectomy).

Patients were ineligible if they had inoperable tumors, true inflammatory carcinoma, or were unwilling to undergo mastectomy at the end of neoadjuvant treatment.

Patients were not permitted any previous exposure to tamoxifen or other selective ER modulators, or treatment with a non-approved or experimental drug at 3 months prior to randomization.

For those centers that used neoadjuvant chemotherapy, specification of the chemotherapy regimen was performed before any patients were randomized. Only one chemotherapy regimen was allowed for all patients randomized from a single center and was chosen from commonly used worldwide regimens.

The use of chemotherapy and/or radiotherapy in the adjuvant period of the study was allowed. If given, the treatment followed the standard patient-management procedures in use at the investigational site.

Trial design

This was a randomized, double-blind, double-dummy study evaluating the efficacy of anastrozole versus tamoxifen as neoadjuvant therapy for postmenopausal women with large, operable or potentially operable, locally advanced, hormone receptor-positive, invasive breast tumors. Patients were randomized on a 1:1 basis to receive either anastrozole (1 mg daily p.o.) or tamoxifen (20 mg daily p.o.), with or without chemotherapy and/or radiotherapy for 12 weeks before primary surgery, and they were to continue receiving study medication for 5 years or until recurrence, intolerable toxicity, or patient refusal. In the rare event of progression during the neoadjuvant part of the trial, patients were withdrawn from study treatment and further treatment, including surgery, was at the investigator's discretion.

The primary objectives of this study were to assess the histopathological response and consistency of histopathological and clinical tumor response following neoadjuvant study treatment. Secondary objectives were to summarize the changes in ER and PgR status between baseline and surgery. Adverse events, monitored as part of the main PROACT trial, are reported elsewhere (Cataliotti et al. 2006).

This study was conducted in accordance with the principles specified in the Declaration of Helsinki and was consistent with International Conference on Harmonization/ Good Clinical Practice requirements. All patients gave written and informed consent.

Assessments

The longest diameter of the tumor was recorded at baseline and at 3 months using ultrasound. In order to avoid operator variability and improve reproducibility, the ultrasound measurements were determined to be performed by the same doctor before and after treatment (i.e. at baseline and 3 months). Although RECIST criteria suggest that ultrasound should not be used to assess tumor response, except in operable and locally advanced breast cancer, it has been shown that ultrasound measurement provides the closest correlations with overall pathological measurements of primary breast tumors (Dixon et al. 1999). Therefore, ultrasound measurements were used in this study.

The objective tumor response at 3 months was defined as a CR (disappearance of the tumor) or partial response ($\geq$30% decrease from baseline in the largest diameter of the tumor), according to the World Health Organization Response Evaluation Criteria in Solid Tumors (RECIST) (World Health Organization 2002).

The histopathological response was assessed by comparing the pathological specimen collected at baseline (via needle biopsy) with that collected at 3 months (from excised tumors). The response was evaluated using the Pathological Response Criteria for Breast Cancer as defined by the Japanese Breast Cancer Society (Table 1) (Kurosumi et al. 2001). Central evaluation was performed by three pathologists, and final judgment was confirmed by consensus. All evaluations of pathological effects were performed blinded to information on clinical response and treatment received.

ER and PgR status using tissue samples obtained at baseline and at 3 months was assessed using an immunohistochemical method, where a 10% or more staining of the nucleus of a cancer cell is categorized as positive.

Statistical analysis

The intent-to-treat (ITT) population for histopathological assessment included all randomized Japanese patients (living in Japan). The per-protocol population of all major protocol violators and deviators, and patients with negative ER and PgR status excluded from the ITT population at baseline, as assessed by the Central Pathological Review Committee.

The histopathological response rate was calculated with two-sided 95% CI for each treatment group. No formal treatment comparison between anastrozole and tamoxifen was performed.

Table 1 Classification of response criteria

Grade	Response criteria
0	*No response.* Almost no change in cancer cells following treatment
1a	*Mild response.* Mild changes[a] in cancer cells regardless of the area, or marked changes[b] seen in <1/3 cancer cells
1b	*Moderate response.* Marked changes in ≥1/3 but <2/3 tumor cells
2	*Marked response.* Marked changes in ≥2/3 tumor cells
3	*Complete response.* Necrosis or disappearance of all tumor cells. Replacement of all cancer cells by granuloma-like and/or fibrous tissue. In the case of complete disappearance of cancer cells, pretreatment pathological evidence of the presence of cancer was necessary

When response assessment of intraductal components and lymph-node metastases is necessary, the above-mentioned criteria should be used and the assessment result for the intraductal components (d) and lymph-node metastases (n) can be added to the result of assessment of primary lesion response, e.g. Grade 1a + 1b(d) + 2(n)

Biopsy specimens (obtained by needle biopsy or incisional biopsy) should not be used for the final response assessment; but histopathological findings of individual specimens should be described

If the therapeutic response ranges over two grades, the lower grade of response should be selected

For Grade 3 assessment, multiple specimens must be examined

[a] Mild changes include slight degenerative changes in cancer cells not suggestive of the death of cancer cells (including cancer cells with vacuolation of cytoplasm, eosinophilic cytoplasm, and swelling of the nucleus, etc.)

[b] Marked changes include marked degenerative changes in cancer cells, suggesting that the cancer cells should barely survive (including liquefaction, necrosis, and disappearance of the cancer cells)

Results

Patients

A total of 97 patients, enrolled from 25 Japanese centers, were randomized to receive anastrozole ($n = 48$) or tamoxifen ($n = 49$) (Fig. 1). Of these patients, 43 in the anastrozole group and 39 in the tamoxifen group received endocrine therapy alone in the neoadjuvant phase of the study. The remaining 15 patients received chemotherapy as well as endocrine treatment. The recruited patient population was representative of the target population of postmenopausal women with large, operable or potentially operable, locally advanced, ER-positive and/or PgR-positive breast cancer. The two treatment arms were well balanced with respect to patient characteristics and demographics (Table 2).

Histopathological response

In the ITT population, the histopathological response rate following preoperative anastrozole treatment was numerically greater than that of the following preoperative tamoxifen. A total of 17/48 patients receiving preoperative anastrozole treatment had tumors of Grade ≥1b, corresponding to a histopathological response rate of 35.4% (95% CI 22.2, 50.5). This finding compared with 6/49 patients receiving preoperative tamoxifen, a histopathological response rate of 12.2% (95% CI 4.6, 24.8) (Fig. 2). For patients who did not receive any chemotherapy during preoperative anastrozole therapy, 16/43 patients had responses of Grade ≥1b (a histopathological response rate of 37.2%) compared with 3/39 patients treated with preoperative tamoxifen who did not receive chemotherapy (a histopathological response rate of 7.7%; Fig. 3). The endocrine-therapy-only subgroup was not pre-specified and is, therefore, included for descriptive purposes only.

There were no recorded histopathological CRs in either study arm. As in the ITT population, histopathological response rates for the per-protocol population were numerically greater in the anastrozole group than in the tamoxifen group; histopathological response rates of 37.0% (95% CI 23.2, 52.5) and 13.6% (95% CI 5.2, 27.4) were calculated for patients receiving anastrozole and tamoxifen, respectively. For patients in the per-protocol population who did not receive preoperative chemotherapy, 16/43 patients who received anastrozole had a histopathological response of Grade ≥1b (37.2%), compared with 3/36 patients receiving tamoxifen (8.3%).

Consistency of histopathological and clinical objective tumor response

The comparison of histopathological and clinical objective responses for the ITT population is shown in Table 3. Overall, 63/97 patients (64.9%; 11 responders and 52 non-responders) in the ITT population had consistent histopathological and clinical objective responses. The per-protocol population showed a similar level of consistency, with agreement between histopathological and clinical objective response in 57/90 patients (63.3%; 11 responders and 46 non-responders).

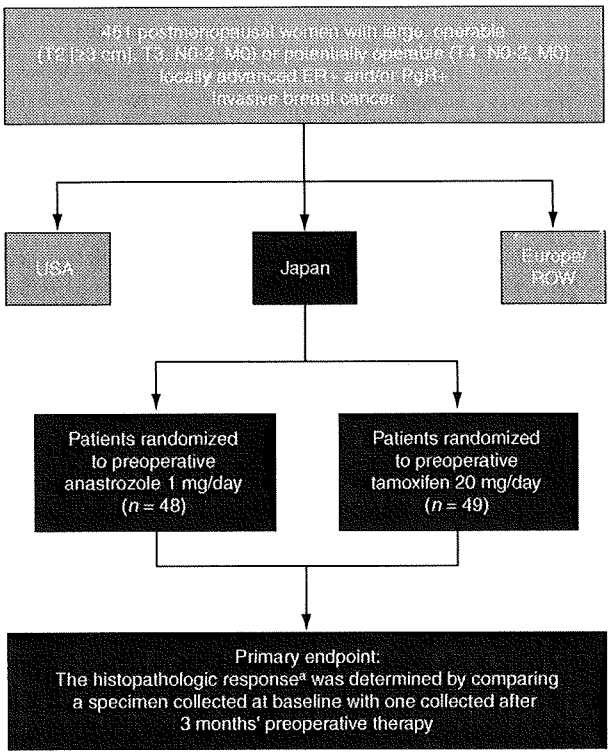

Fig. 1 Patients and study design. [a] The response was evaluated on a 5-grade categorical scale (Grade 0, 1a, 1b, 2, and 3), according to the Japanese Histopathological Criteria for Assessment of Therapeutic Response in Breast Cancer, by the Central Pathological Review Committee. A response was defined as Grade ≥1b (i.e. a degenerative change in ≥1/3 of constituent carcinoma cells). The main outcome was an assessment of the invasion area

Table 2 Patient demographics and baseline disease characteristics

	Anastrozole 1 mg/day (n = 48)	Tamoxifen 20 mg/day (n = 49)
Median age (range), years	61.5 (51.4–84.7)	61.6 (51.4–81.3)
Median body mass index (range), kg/m^2	23.7 (15.7–32.0)	23.6 (18.9–35.2)
Feasible surgery, n (%)		
Breast conserving	2 (4.2)	2 (4.1)
Mastectomy	46 (95.8)	47 (95.9)
Median tumor dimension (range), cm		
By ultrasound	3.6 (1.7–9.0)	3.6 (1.8–6.3)
By caliper	4.2 (3.0–11.5)	4.5 (3.0–11.0)
TNM classification, n (%)		
T2	31 (64.6)	26 (53.1)
T3	9 (18.8)	15 (30.6)
T4a	0 (0)	0 (0)
T4b	8 (16.7)	8 (16.3)
N0	32 (66.7)	32 (65.3)
N1	15 (31.3)	14 (28.6)
N2	1 (2.1)	3 (6.1)
M0	48 (100.0)	49 (100.0)
ER and PgR status		
ER+ and PgR+	32 (66.7)	27 (55.1)
ER+ and PgR–	15 (31.3)	21 (42.9)
ER– and PgR+	1 (2.1)	1 (2.0)
Hormonal therapy only	43 (89.6)	39 (79.6)

ER estrogen receptor, *PgR* progesterone receptor, *TNM* Tumor, node, metastases

Change in hormone-receptor status

In the ITT population, a total of 40 patients who received anastrozole and 37 who received tamoxifen had an ER-positive status at baseline (Table 4). Of these patients, the ER status of 5 patients who received anastrozole changed to negative compared with 20 patients who received tamoxifen at 3 months. The ER status of the remaining patients (anastrozole, n = 35; tamoxifen, n = 17) remained positive at 3 months.

In the ITT population at baseline, 17 patients who received anastrozole and 11 patients who received tamoxifen were PgR-positive (Table 4). At 3 months, the PgR status of 16 patients who received anastrozole changed to negative when compared with only one patient who received tamoxifen.

The change in ER/PgR status in the per-protocol population was very similar to the results of the ITT population in both treatment groups (data not shown).

Discussion

A numerically higher histopathological response rate was observed following preoperative treatment with anastrozole than following preoperative tamoxifen in both the ITT and per-protocol populations. Histopathological response was consistent with the clinical response in approximately two-thirds of cases in both the ITT and per-protocol populations.

The histopathological assessment criteria used in this study were originally designed for the assessment of the efficacy of cytotoxic chemotherapeutic agents. Here, these criteria have been used to describe therapeutic responses of lesser magnitude than pCR with endocrine agents, which are better tolerated than chemotherapy. The histopathological response rate of 35% (37.2% in patients who did not receive preoperative chemotherapy) with anastrozole concurs with the clinical objective response rate (by RECIST criteria) and can, therefore, be considered reliable.

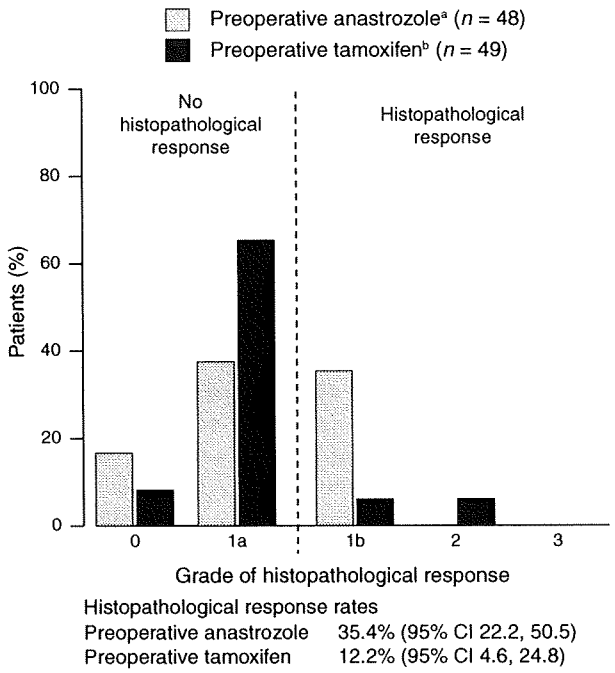

Fig. 2 Histopathological response at 3 months for the intent-to-treat population. [a]Five patients in the anastrozole group discontinued preoperative treatment. [b]Six patients in the tamoxifen group discontinued preoperative treatment, and one patient did not receive any study treatment

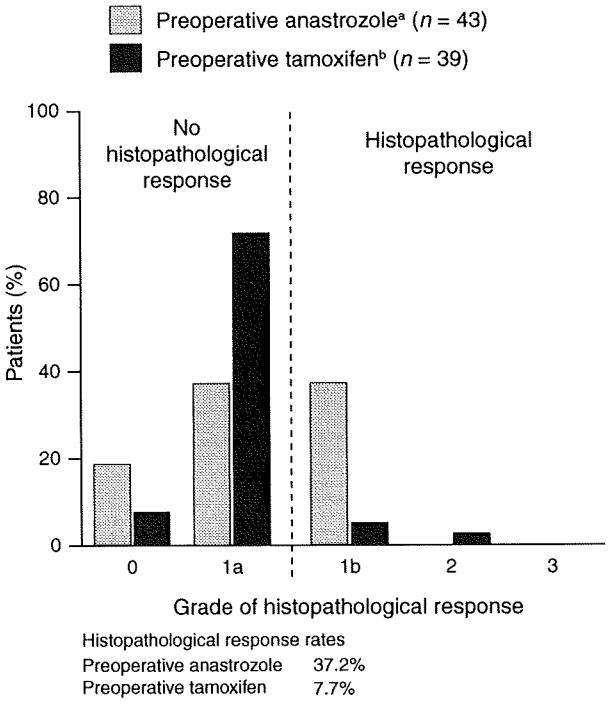

Fig. 3 Histopathological response at 3 months for patients in the intent-to-treat population who did not receive preoperative chemotherapy. [a] Three patients in the anastrozole group discontinued preoperative treatment. [b] Four patients in the tamoxifen group discontinued preoperative treatment, and one patient did not receive any study treatment

In this study, the results of pathological evaluation were consistent with ultrasonographic findings in 63/97 patients (64.9%), and not consistent in 34 (≈35%) patients. It is well known that the clinical response may not be completely consistent with the pathological one (Beresford et al. 2007); however, pCR is used as the endpoint of many preoperative treatments. The degree of therapeutic response evaluated by histological examination can differ from that of based on ultrasonographic findings due to various factors, two examples follow. The therapeutic response based on ultrasonographic findings is lower when cancer cells at the center of the mass disappear and are replaced by fibrous elements (Thomas et al. 2007), but marginal cancer cells still form a ring; in this example the histological response is evaluated higher than the ultrasonographic one. The therapeutic response based on ultrasonographic findings is higher when the treatment is effective on intraductal components of cancer with a significant decrease in the overall volume of the mass, but it is hardly effective on small foci of invasion (Matsuo et al. 2002); in this example the histological response is evaluated lower than the ultrasonographic one. Further follow-up is required to establish an association between clinical or histopathological response and treatment outcome, as it has been established for neoadjuvant chemotherapy.

Table 3 Cross-tabulation of histopathological and objective tumor responses in the intent-to-treat patient population

Histopathological response	Clinical objective tumor response		
	Responder	Non-responder	Total
Responder	11	12	23
Non-responder	21	52	73[a]
Not collected[b]	0	1	1
Total	32	65[a]	97

[a] Eleven patients withdrew before 3 months and are included with the non-responders

[b] Patient did not receive any study treatment

Considering that adjuvant endocrine therapy is typically administered over a period of 5 years, and that endocrine therapy in effect starves tumor cells of estrogenic stimulation rather than directly eliminating them, a period of 12 weeks may be insufficient to gain a pCR with neoadjuvant endocrine treatment. This factor may account for the lack of pCRs in our patients. The prognostic significance of a moderate response (i.e. a cCR or partial response, but not a pCR) has yet to be established for neoadjuvant endocrine therapy, but will be defined with longer follow-up.

Table 4 Change in estrogen/progesterone-receptor status by histopathological assessment in the intent-to-treat patient population

Baseline	Post-observation		
	Positive	Negative	Not evaluated
ER status			
Anastrozole (n = 48)			
Positive (n = 40)	35 (72.9)	5 (10.4)	0 (0.0)
Negative (n = 3)	2 (4.2)	1 (2.1)	0 (0.0)
Not evaluated (n = 5)	0 (0.0)	0 (0.0)	5 (10.4)
Tamoxifen (n = 49)			
Positive (n = 37)	17 (34.7)	20 (40.8)	0 (0.0)
Negative (n = 5)	1 (2.0)	4 (8.2)	0 (0.0)
Not evaluated (n = 7)[a]	0 (0.0)	0 (0.0)	7 (14.3)[a]
PgR status			
Anastrozole (n = 48)			
Positive (n = 17)	1 (2.1)	16 (33.3)	0 (0.0)
Negative (n = 26)	0 (0.0)	26 (54.2)	0 (0.0)
Not evaluated (n = 5)	0 (0.0)	0 (0.0)	5 (10.4)
Tamoxifen (n = 49)			
Positive (n = 11)	10 (20.4)	1 (2.0)	0 (0.0)
Negative (n = 31)	6 (12.2)	25 (51.0)	0 (0.0)
Not evaluated (n = 7)[a]	0 (0.0)	0 (0.0)	7 (14.3)[a]

[a] Six patients discontinued the neoadjuvant phase and one patient did not receive any study treatment

These results support the clinical findings from the main PROACT trial, in which preoperative anastrozole produced numerically superior objective response rates compared with preoperative tamoxifen, and highlight the value of preoperative endocrine therapy with aromatase inhibitors for postmenopausal women with large, operable or potentially operable breast tumors (Cataliotti et al. 2006). Significantly higher objective response rates compared with tamoxifen have been obtained with preoperative letrozole (20 and 30% for tamoxifen and letrozole, respectively; $P = 0.0006$), and ongoing preoperative endocrine studies should elucidate receptor cross-talk and endocrine resistance pathways further (Dixon et al. 2002; Smith (2003); Huober et al. (2004).

Preliminary evidence suggests that hormone receptor-positive patients experience weaker responses to neoadjuvant chemotherapy than hormone receptor-negative patients (pCR rate of 5.0 and 20.6%, respectively) (Buzdar et al. 2003). Recently published data indicate that neoadjuvant chemotherapy that includes trastuzumab [a monoclonal antibody for the human epidermal growth-factor receptor (HER) 2 protein], for the treatment of patients with HER2-positive disease, elicits significantly higher rates of pCR than chemotherapy alone (25 and 66.7% for chemotherapy and chemotherapy plus trastuzumab, respectively; $P = 0.02$) (Buzdar et al. 2005). Although based on a small number of patients (n = 42), these data illustrate that better responses may be produced by tailoring neoadjuvant therapy to individual tumor type.

Histopathological assessment of changes in ER/PgR status suggests that anastrozole therapy may induce a negative PgR change, whereas, tamoxifen therapy may induce a negative ER change. Although the mechanism of these changes has not been clarified; this result is consistent with other studies with aromatase inhibitors (Miller et al. 2001, (2003). In the first of these studies, for PgR was reduced in 16/17 PgR-positive cancers treated with anastrozole and in all 21 positive cancers treated with the aromatase inhibitor, letrozole (Miller et al. 2001). Likewise, in the second of these studies, PgR reactivity was reduced in 20/21 evaluable cases treated with letrozole, becoming undetectable in 16 patients. Only marginal changes were observed in ER expression following letrozole therapy, but following treatment with tamoxifen, ER expression was markedly reduced in most cases (Miller et al. 2003).

In conclusion, these data support the efficacy and tolerability of neoadjuvant endocrine therapy in patients with hormone receptor-positive disease shown in the PROACT trial. Neoadjuvant anastrozole produced a numerically greater response rate compared with neoadjuvant tamoxifen. The histological response to therapy showed some correlation with the observed clinical response, although further follow-up is required to confirm a subsequent effect on survival.

Acknowledgments Masami Ogita, Motoshi Tamura, Organization Hokkaido Cancer Center; Morihiko Kimura, Gunma Cancer Center; Mitsuru Miyauchi, Naohito Yamamoto, Chiba Cancer Center; Toshio Tabei, Saitama Cancer Center; Toshiaki Saeki, National Cancer Center Hospital East; Yasuhiro Fujiwara, National Cancer Center Hospital; Seigo Nakamura, St Luke's International Hospital; Taro Asaga, Masaaki Inaba, Kanagawa Cancer Center; Yutaka Tokuda, Hospital of the Tokai University; Muneaki Sano, Niigata Cancer Center Hospital; Shigeto Miura, Hiroji Iwata, Aichi Cancer Center; Hirotaka Iwase, Hiroko Yamashita, Nagoya City University Hospital; Tooru Takeuchi, Marumo Hospital; Masahiro Watatani, Hospital of Kinki University; Eisei Shin, Toshimasa Tsujinaka, Norikazu Masuda, National Hospital Organization Osaka National Hospital; Kazuya Miyoshi, National Hospital Organization Fukuyama Medical Center; Kenjiro Aogi, Toshiaki Saeki, National Hospital Organization Shikoku Cancer Center; Shoshu Mitsuyama, Kita-kyushu Municipal Medical Center; Shinji Ohno, National Hospital Organization Kyushu Cancer Center; Yuzo Hamada, Fumie Matsuo, Hospital of Fukuoka University; Reiki Nishimura, Kumamoto City Hospital; Hiroaki Ueo, Ryoji Abe, Oita Prefectural Hospital. Institution is where the study was conducted. We also thank Martin Quinn PhD, who provided medical writing support. The PROACT study was funded by AstraZeneca.

Conflict of interest The authors state that they have no conflicts of interest to declare.

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Breast Cancer (2008) 15:121–132
DOI 10.1007/s12282-008-0032-5

CONFERENCE PAPER

Presidential symposium: Individualized diagnosis for tailored treatment of breast cancer

Individualization of breast cancer based on histopathological features and molecular alterations

Hitoshi Tsuda

Published online: 5 March 2008

Abstract Histopathological findings and molecular alterations well reflect the biological properties of individual primary breast carcinomas. Specifically, pT (size of the invasive component), pN (number of metastatic lymph nodes), histological or nuclear grade, lymphovascular invasion, hormone receptors, and *HER2* (c-erbB-2) gene overexpresison or amplification are known to be effective markers for assessing the risk of operable primary breast carcinoma, albeit incompletely. It is expected that additional molecular markers and novel diagnostic tools will be developed in the future to facilitate a more accurate characterization of higher risk node-negative breast carcinomas.

Keywords Basal-like type · Grade · Lymph node metastasis · Prognostic factor · Tumor size

Abbreviations

CGH	Comparative genomic hybridization
CK	Cytokeratin
DCIS	Ductal carcinoma in situ
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
HE	Hematoxylin and eosin
IHC	Immunohistochemistry
ITC	Isolated tumor cells
LCIS	Lobular carcinoma in situ
ly	Lymphatic invasion
NCCN	National Comprehensive Cancer Network
pCR	Pathological complete response
PgR	Progesterone receptor
PST	Primary systemic therapies
SLN	Sentinel lymph node
SNNS	Sentinel lymph node navigation surgery
v	Vascular invasion

This article is based on a presentation delivered at Presidential Symposium 1, "Breast cancer: individualized diagnosis for tailored treatment," held on 29 June 2007 at the 15th Annual Meeting of the Japanese Breast Cancer Society in Yokohama.

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Introduction

Breast cancers show considerable variation in terms of their histological features and molecular alterations, and such factors are known to influence patient outcome and tumor clinical behavior. Prognostic factors of breast cancer can be largely categorized into factors related to (1) the extent of (macro- and microscopically visible) tumor spread, (2) biological properties of the cancer cells, and (3) host–tumor relationship (Fig. 1). Factors related to the extent of tumor spread include clinical stage, size of the invasive cancer component, and the status of regional lymph node metastasis and distant metastasis. Those related to the biological properties of cancer cells, which account for differences in prognosis among patients with the same extent of tumor spread, are histological or nuclear grade of the cancer cells, lymphovascular permeation, and *HER2* (HER2/*neu*, c-erbB-2) overexpression and/or gene amplification. Hormone receptor status is used mainly to identify patients eligible for preoperative or postoperative endocrine therapies, but estrogen receptor (ER) and progesterone receptor (PgR) status is also prognostically

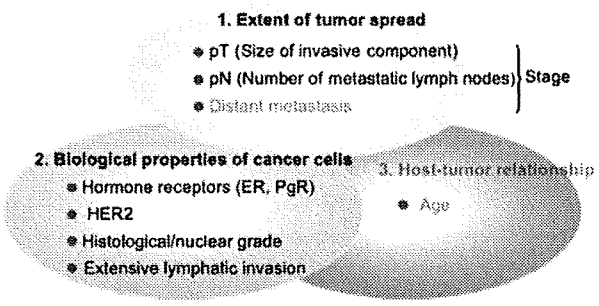

Fig. 1 Prognostic factors of breast cancer. These can be largely categorized into factors of: *1* the extent of tumor spread, *2* biological properties if cancer cells, 3 host-tumor relationship

independent of the extent of tumor spread. Factors related to the host–tumor relationship are patient age and immune status, although these are not well characterized.

In patients with operable breast cancer, these factors are now used routinely for evaluating the prognosis and predicting the response of cancer cells to specific therapeutic drugs. At the St Gallen International Conference in 2007, the use of adjuvant chemotherapies was recommended based on a combination of the above-mentioned factors (Tables 1, 2) [1]. There is a consensus that node-metastasis-positive breast cancers should be treated with systemic adjuvant chemotherapy. In node-metastasis-negative (pN0) breast cancer, risk estimation is based on a combination of age, hormone receptors, size of the invasive tumor component, grade, lymphovascular invasion, and *HER2* expression [1–3]. In the National Comprehensive Cancer Network (NCCN) guidelines, the same factors are utilized for evaluating the risk of primary breast cancer [3].

This article provides an overview of prognostic and predictive factors that are used routinely for designing individual therapies for patients with breast cancer. Because the present classifications of pN0 breast cancers into groups of intermediate and low risk are still insufficient, we also review potentially useful biomarkers or tests that allow more a precise prognostication.

Size of invasive component of primary tumor (pT factor)

In the *TNM Classification of malignant tumours*, 6th edn [4], the size of an invasive primary tumor is classified into pT0, pTis, pT1, pT2, pT3, and pT4 (pT = primary tumor; Table 3). pTis is non-invasive carcinoma and is usually stage 0. According to the histological classification listed in the *General rules for clinical and pathological recording of breast cancer*, 15th edn [5] (abbreviated as "general rules" hereafter), breast carcinomas are classified into 17 histological types, two types of non-invasive carcinoma, three common types of invasive ductal carcinoma, and 11 special types, including Paget's disease. Among these, non-invasive carcinomas and several histological types are of clinical significance. Non-invasive carcinomas, such as non-invasive ductal carcinoma, or ductal carcinoma in situ (DCIS), non-invasive lobular carcinoma or lobular carcinoma in situ (LCIS), and Paget's disease without invasion, are important because the prognosis of patients with these types of breast cancer is excellent. Among the special types, mucinous carcinoma, medullary carcinoma, adenoid cystic carcinoma, and tubular carcinoma are known to show a good clinical outcome.

pT1 is subdivided into pT1mic, pT1a, pT1b, and pT1c when the diameter of the invasive component of the primary tumor is $\leq$ 0.1 cm, >0.1–0.5 cm, >0.5–1.0 cm, and >1.0–2.0 cm, respectively. In the NCCN guidelines, the risk of recurrence is estimated to differ among pT1a, pT1b, and pT1c [3]. In the St Gallen meeting consensus, the size of the invasive tumor is classified into categories of $\leq$ 2.0 and >2.0 cm, although some panel members consider pT1a and pT1b (i.e., pT $\leq$1 cm) tumors that are node-negative to

Table 1 Definition of risk categories for patients with operable breast cancer (reproduced from [1] with modifications)

Risk category	Parameters
Low risk	Node negative AND all of the following features:
	pT $\leq$2 cm, and Grade 1, AND
	Absence of extensive peritumoral vascular invasion, AND
	ER and/or PgR expression, AND
	HER2 gene neither overexpressed nor amplified, AND
	Age $\geq$35 years
Intermediate risk	Node negative AND at least one of the following features:
	pT >2 cm, or Grade 2–3, OR
	Presence of extensive peritumoral vascular invasion, OR
	ER and PgR absent, OR
	HER2 gene overexpressed or amplified, OR
	Age <35 years
	Node positive (1–3 involved nodes) AND
	ER and/or PgR expressed, AND
	HER2 gene neither overexpressed nor amplified
High risk	Node positive (one to three involved nodes) AND
	ER and PgR absent, OR
	HER2 gene overexpressed or amplified
	Node positive (four or more involved nodes)

pT Primary tumor, *PgR* progesterone receptor, *ER* estrogen receptor