III.　研究成果の刊行に関する一覧表

別紙4

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
津田均	Basic 5 病理診断—分子生物学的アプローチ(臓器特異的遺伝子異常を用いた原発巣の推定)	向井博文	原発不明がん適切な診断・治療のポイント	メジカルビュー社	大阪	2012	46-53
津田均	Advance 3 病理診断—遺伝子変異の検出の意義(例えばEGFR, KRAS, ARID1A, PIK3CA, BRAF, p53などの変異、HER2, EGFR増幅、染色体欠失、染色体転座など)	向井博文	原発不明がん適切な診断・治療のポイント	メジカルビュー社	大阪	2012	164-170

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Iwata, H., Tsuda, H., et al.	Docetaxel followed by fluorouracil/epirubicin/ cyclophosphamide as neoadjuvant chemotherapy for patients with primary breast cancer	Jpn. J. Clin. Oncol.	41(7)	867-875	2011
Ueda, S., Tsuda, H., et al.	Early metabolic response measured by FDG PET/CT predicts cell-cycle response to neoadjuvant letrozole in patients with hormone receptor-positive primary breast cancer. A pilot study.	Breast Cancer	18(4)	299-308	2011
Onoe, S., Tsuda, H., et al.	Feasibility of breast conserving surgery for Paget's disease.	Breast	20(6)	515-518	2011
Ono, M., Tsuda, H., et al.	Tumor-infiltrating lymphocytes are correlated with response to neoadjuvant chemotherapy in triple-negative breast cancer.	Breast Cancer Res. Treat.	132(3)	793-805	2012
Yoshida, M., Tsuda, H., et al.	Loss of heterozygosity on chromosome 16q suggests malignancy in core needle biopsy specimens of intraductal papillary breast lesions.	Virchows Arch.	460(5)	497-504	2012

IV. 研究成果の刊行物・別刷

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2011;41(7)867–875
doi:10.1093/jjco/hyr081

Docetaxel Followed by Fluorouracil/Epirubicin/Cyclophosphamide as Neoadjuvant Chemotherapy for Patients with Primary Breast Cancer

Hiroji Iwata[1,*], Nobuaki Sato[2], Norikazu Masuda[3], Seigo Nakamura[4], Naohito Yamamoto[5], Katsumasa Kuroi[6], Masafumi Kurosumi[7], Hitoshi Tsuda[8], Futoshi Akiyama[9], Yasuo Ohashi[10] and Masakazu Toi[11]

[1]Department of Breast Oncology, Aichi Cancer Center Hospital, Aichi, [2]Department of Surgery, Niigata Cancer Center Hospital, Niigata, [3]Department of Surgery, National Hospital Organization Osaka National Hospital, Osaka, [4]Breast Surgical Oncology, St Luke's International Hospital, Tokyo, [5]Division of Breast Surgery, Chiba Cancer Center, Chiba, [6]Division of Clinical Trials and Research and Department of Surgery, Tokyo Metropolitan Cancer and Infectious Disease Center, Komagome Hospital, Tokyo, [7]Department of Pathology, Saitama Cancer Center, Saitama, [8]Division of Clinical Laboratory, National Cancer Center Hospital, [9]Division of Clinical Pathology, Cancer Institute of the Japanese Foundation for Cancer Research, [10]Department of Biostatistics/Epidemiology and Preventive Health Science, School of Health Science and Nursing, University of Tokyo, Tokyo and [11]Department of Surgery (Breast Surgery), Graduate School of Faculty of Medicine, Kyoto University, Kyoto, Japan

*For reprints and all correspondence: Hiroji Iwata, Department of Breast Oncology, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan. E-mail: hiwata@aichi-cc.jp

Received January 21, 2011; accepted April 24, 2011

Objective: This multicenter, open-label, single-arm, Phase II study assessed the efficacy of a neoadjuvant chemotherapy with docetaxel (75 mg/m^2 q3w) followed by 5-fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2 and cyclophosphamide 500 mg/m^2 q3w in patients with early-stage breast cancer.

Methods: Women with resectable breast cancer (T1c–3 N0 M0 or T1–3 N1 M0) were enrolled. Before surgery, patients received four cycles of docetaxel followed by four cycles of 5-fluorouracil, epirubicin, and cyclophosphamide. The primary endpoint was the pathological complete response (pCR) rate defined for the breast alone, assessed by a central review committee. Secondary endpoints included clinical response and safety.

Results: One hundred and thirty-seven patients were enrolled. Of the 132 patients assessable for pathologic response, 23% (95% confidence interval, 16–31%) experienced a pathological complete response and 6% (95% confidence interval, 3–12%) had a near pathological complete response (few remaining cancer cells), resulting in a quasi-pathological complete response of 29% (95% confidence interval, 21–37%). Clinical response rate following the initial docetaxel regimen was 64%. The overall clinical response rate after completion of 5-fluorouracil, epirubicin, and cyclophosphamide was 79%; breast-conserving surgery was performed in 79% of patients. More patients with triple-negative disease (estrogen/progesterone receptors negative; human epidermal growth factor 2 negative) experienced a pathological complete response [14/29, (48%); 95% confidence interval, 29–68%] versus those with other molecular subtypes. The safety profile was acceptable.

Conclusions: Eight cycles of neoadjuvant chemotherapy—docetaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide—are tolerable and conferred high rates of pathological complete response and breast-conserving surgery. Patients with triple-negative disease were more likely to achieve pathological complete response versus other subtypes, suggesting that selecting appropriate neoadjuvant chemotherapy based on molecular subtype could be possible.

Key words: breast neoplasms – neoadjuvant therapy – FEC protocol – docetaxel

INTRODUCTION

Neoadjuvant chemotherapy has been widely used for patients with operable breast cancer to increase the chance of breast conservation (1–7). Furthermore, response to neoadjuvant treatment can provide important information on long-term survival outcomes. Pathological complete response (pCR) in the breast and axillary lymph nodes predicts a favorable prognosis, whereas a lack of pCR in the breast and node-positive status do not (6,7). This implies the possibility of tailoring subsequent treatment according to the response to initial treatment (7–12). In addition, correlative studies of tumor samples before and after treatment may provide information on markers that could predict response or resistance to treatment (13–16).

Results from the National Surgical Adjuvant Breast and Bowel Project (NSABP) Protocol B-18 trial demonstrated the impact of neoadjuvant chemotherapy in patients with operable early-stage breast cancer (17). The protocol-specified anthracycline-containing regimen—four cycles of doxorubicin and cyclophosphamide (AC)—resulted in an increased likelihood of breast-conserving surgery (BCS) compared with no neoadjuvant chemotherapy. The study established pCR as a prognostic marker for long-term disease-free survival (DFS) and demonstrated that there was no difference in survival if chemotherapy was administered before or after surgery. Subsequent studies, such as the Aberdeen trial, have demonstrated the benefit of the sequential addition of taxanes to neoadjuvant anthracycline regimens (5). The NSABP Protocol B-27 trial demonstrated that, compared with neoadjuvant AC alone, the addition of sequential docetaxel doubled the pCR rate, increased the clinical complete response rate (RR) and increased the proportion of patients with negative axillary nodes (7–18).

We previously conducted a Phase II study to evaluate the clinical and pathological response and safety of the FEC regimen (5-fluorouracil, epirubicin and cyclophosphamide) followed by docetaxel as neoadjuvant chemotherapy in Japanese women with early-stage breast cancer [Japan Breast Cancer Research Group (JBCRG) 01 trial]. The results of this study have been reported previously (19). Although the pCR rate was 16% and BCS was possible for 85% of patients, there were some safety concerns, with 18% of patients experiencing febrile neutropenia and 41% of patients experiencing Grade 1/2 peripheral edema (no Grade 3/4 events observed) following the docetaxel regimen (unpublished data). Disease progression occurred in 6% of patients after the completion of all planned treatment (unpublished data).

In an effort to achieve a higher pathological RR with an improved safety profile, we decided to evaluate the efficacy and safety of docetaxel followed by FEC (JBCRG 03 trial)—the reverse of the sequence of chemotherapy used in the JBCRG 01 trial (19). The clinical and pathological effects and the toxicity profile of this regimen are presented here, and the results of predictive marker analyses are discussed.

PATIENTS AND METHODS

Patient Eligibility

This was a multicenter, open-label, single-arm, Phase II study that recruited patients via central registration. Japanese women aged 20–59 years with histologically proven early-stage breast cancer (T1c–3 N0 M0 or T1–3 N1 M0) were enrolled. No prior chemotherapy, radiotherapy, hormonal therapy or immunotherapy was allowed. Other inclusion criteria were Eastern Cooperative Oncology Group performance status 0–1; white blood cell count 4000–12 000/mm^3; neutrophil count $\geq$ 2000/mm^3; platelet count $\geq$ 100 000/mm^3; hemoglobin $\geq$ 9.5 g/dl; serum bilirubin $\leq$ 1.25 times upper limit of normal (ULN); creatinine $\leq$ 1.5 times ULN and aspartate aminotransferase and alanine aminotransferase $\leq$ 1.5 times ULN. Patients with congestive heart failure or left ventricular ejection fraction $\leq$60% were excluded. Patients were also excluded if they had confirmed infection; serious concomitant illness such as severe cardiovascular disease, uncontrolled diabetes, malignant hypertension or hemorrhagic disease; active concomitant malignancy; brain metastasis; peripheral neuropathy; history of edema with severe drug allergy; or previous long-term corticosteroid therapy. Pregnant or lactating women were excluded. Mammography, ultrasonography, magnetic resonance imaging or computed tomography was used to assess the presence of tumors. Baseline evaluations included complete blood cell and platelet count, routine blood chemistry and liver function tests, chest X-ray, bone scan, electrocardiogram and echocardiogram.

The local ethics committee or institutional review board approved the study at each institution. All patients gave written informed consent to participate. The protocol was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

Treatment

Four cycles of docetaxel (75 mg/m^2) administered intravenously (i.v.) every 21 days were followed by four cycles of FEC (5-fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2 and cyclophosphamide 500 mg/m^2) administered i.v. on Day 1 every 21 days before surgery. Premedication was administered based upon each physician's decision to prevent edema, nausea and allergic reactions (e.g. dexamethasone 12 mg i.v. and/or granisetron 4 mg i.v. on Day 1, and oral dexamethasone 8 mg on Days 2 and 3 of docetaxel treatment; dexamethasone 24 mg i.v. on Day 1 and oral dexamethasone 8 mg on Days 2–6 with the FEC regimen). Administration of granulocyte colony-stimulating factor and antibiotics was left to the judgment of each investigator.

Clinical Response Assessment

Tumor assessments were performed within 4 weeks before docetaxel treatment, after completion of docetaxel treatment

and before surgery. Tumor response was assessed using the modified Response Evaluation Criteria in Solid Tumors guidelines (in which confirmatory scans/assessments were not required due to the timing of surgery), for patients who had measurable lesions.

Central Pathologic Assessment

Hematoxylin and eosin-stained slides were prepared from core needle biopsy and surgical specimens from the primary tumor. All surgical specimens were cut in 5 mm interval and all surfaces were microscopically examined in each institution. Pathological response of chemotherapy was assessed by a central review committee consisting of three pathologists who used criteria established by the Japanese Breast Cancer Society. pCR was defined as necrosis and/or disappearance of all tumor cells, and/or the replacement of cancer cells by granulation and/or fibrosis. If only ductal components remained, the pathological response was described as a pCR. Near pCR was defined as extremely high grade marked changes approaching a complete response, with only a few remaining isolated cancer cells (19). Quasi-pCR (QpCR) was the total of both pCR and near pCR. The central review committee evaluated the pathological responses independently from local pathologists. This committee was blinded to the local pathologists' reports. Patients who did not have surgery because of disease progression were considered not to have a pCR.

Hormone Receptor and Human Epidermal Growth Factor 2 Overexpression

Estrogen receptor (ER) and progesterone receptor (PgR) status was determined by immunohistochemistry (IHC) before docetaxel treatment at each participating institute.In general, tumors with more than 10% positively stained tumor cells were classified as positive for ER and PgR. The human epidermal growth factor 2 (HER2) status of the tumor was also determined at each institute by IHC or by fluorescence *in situ* hybridization (FISH) analysis. HER2-positive tumors were defined as those scoring 3+ with IHC staining or testing positive by FISH. HER2-negative tumors were defined as those scoring 0−1+ with IHC or scoring 2+ with IHC and testing negative by FISH.

Surgery and Radiotherapy

Following chemotherapy and clinical assessment of response, patients underwent surgery. If the tumor was too large or invasive for BCS, a modified radical mastectomy was recommended. Careful pathological assessment of tumor margins was performed in accordance with the Japanese Breast Cancer Society criteria (20). Sentinel lymph node biopsy was performed to confirm disease stage or to avoid surgical axillary dissection. Autologous or heterologous reconstructive surgery was performed depending on the patient's requirements and health status. All patients who underwent BCS were given standard radiotherapy to the remaining ipsilateral breast tissue after surgical recovery. For patients diagnosed as sentinel node negative and thus not requiring axillary dissection; radiotherapy to the axilla was allowed.

Toxicity and Dose Modification

Toxicities were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3) throughout treatment with docetaxel and FEC before surgery. Treatment could be postponed for a maximum of 2 weeks only for severe toxicity. If the adverse event (AE) did not improve during this period, chemotherapy was discontinued and surgery was recommended. Dose reductions were permitted for docetaxel from 75 to 60 mg/m^2 and for epirubicin from 100 to 75 mg/m^2 in cases of febrile neutropenia or Grade 3/4 non-hematologic toxicities, except for nausea, vomiting and fatigue.

Statistical Methods

The primary endpoint was the pCR rate. Before the initiation of the current study, the pCR rate for non-taxane anthracycline regimens ranged from 12.8% (NSABP Protocol B-27) (18) to 15.4% (Aberdeen trial) (5). Previously, we had conducted JBCRG01 trial to evaluate the pCR rate defined for breast disease (19). Therefore, in order to detect improvement in the pCR rate in the same definition of our previous study, a sample of 119 patients was required according to binominal distribution, with a one-sided threshold pCR rate of 12%, an expected pCR rate of 22%, an α error of 5% and a β error of 10%. The target number of patients for recruitment was therefore 119, so assuming that 5% of patients would not be evaluable, we planned to enroll 130 patients. Secondary endpoints included safety, clinical RR, rate of BCS, DFS, overall survival and a subset analysis according to biomarkers. Pathological and clinical RRs were calculated with 95% confidence intervals (95% CIs), with each complete RR based on a binominal distribution. Pathological response was evaluated by hormone receptor status and HER2 status. A multiple logistic regression analysis was performed to examine which factors (menopausal status, tumor size, ER and PgR status, HER2 status and clinical response to docetaxel and FEC) were associated with pCR and QpCR.

RESULTS

Patients Characteristics and Treatment

Enrollment took place from October 2005 through October 2006. One hundred and thirty-seven patients were enrolled. Two patients did not receive study treatment because of early withdrawal of consent; therefore, 135 patients were evaluable for safety and clinical response. These evaluable

Table 1. Patients' characteristics

Characteristic	Value[a]
Number of evaluable[b] patients	135
Age (years)	
Median	46
Range	24–62
Performance status, *n* (%)	
0	133 (99)
1	2 (1)
Menopausal status, *n* (%)	
Premenopausal	94 (70)
Postmenopausal	41 (30)
Clinical tumor stage, *n* (%)	
T1	13 (10)
T2	98 (73)
T3	24 (18)
Clinical nodal stage, *n* (%)	
N0	62 (46)
N1	73 (54)
ER status, *n* (%)	
Positive	86 (64)
Negative	46 (34)
Unknown	3 (2)
PgR status, *n* (%)	
Positive	63 (47)
Negative	70 (52)
Unknown	2 (1)
HER2 status,[c] *n* (%)	
0	21 (16)
1+	63 (47)
2+	20 (15)
3+	31 (23)

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PgR, progesterone receptor.
[a]Percentages may not add up to 100% because of rounding.
[b]Number of patients evaluable for safety and clinical response.
[c]Evaluated by immunohistochemistry.

patients included two patients aged 60 and 62 years (included because their age was not considered to influence the evaluation). Two patients were lost to follow-up before surgery, thus 133 patients were evaluable for surgical response. A total of 132 patients were evaluable for pathological response; one patient was excluded owing to lack of confirmation of invasive carcinoma (following the pathologic central review) due to inadequate samples from core needle biopsy before study treatment.

The patient characteristics are summarized in Table 1. Thirty patients (22%) had triple-negative disease, defined as ER-negative, PgR-negative and HER2-negative primary breast cancer, including one patient who was lost to follow-up before surgery.

Overall, 98 patients (73%) completed the planned eight cycles of treatment without dose reductions or study discontinuation. A total of 115 (85%) and 106 (82%) patients completed all four planned treatment cycles of docetaxel and FEC, respectively; dose reductions were necessary in 9 (7%) and 17 (13%) patients, respectively. The majority of the dose reductions were attributable to toxicities, particularly febrile neutropenia during treatment with FEC (10 versus 2 patients during docetaxel treatment). Dose reductions due to neutropenia were required by three patients each during the docetaxel and FEC regimens. Eleven (8%) and six patients (5%), respectively, discontinued treatment during docetaxel and FEC therapy because of toxicities (five patients discontinued during both regimens) or disease progression (six patients during docetaxel and one patient during FEC). The mean dose intensities were 24.2 and 30.3 mg/m^2/week for docetaxel and epirubicin, respectively.

Toxicities

The incidence of treatment-related AEs is summarized in Table 2. Neutropenia was the most common Grade 3/4 treatment-related AE and was observed in 44% and 60% of patients during docetaxel and FEC therapy, respectively. Overall, 67% and 15% of patients experienced at least one episode of Grade 3/4 neutropenia or febrile neutropenia, respectively. For non-hematologic toxicities of any grade, rash, sensory neuropathy, edema, muscle pain and joint pain occurred more frequently during docetaxel treatment than with FEC. Conversely, the frequency of gastrointestinal symptoms, such as nausea, vomiting and anorexia, was higher with FEC than with docetaxel. The frequency of Grade 1/2 peripheral edema was similar during exposure to docetaxel (33%) and FEC (29%); no patient had Grade 3/4 edema. Grade 3/4 non-hematologic toxicities, including gastrointestinal disturbances, were infrequent during both docetaxel and FEC. No fatal AEs were reported.

Clinical Response to Treatment

The overall clinical RR was 79% (106/135; 95% CI, 71–85%), with a clinical complete RR of 21% (29/135), a partial RR of 57% (77/135) and a disease progression rate of 5% (7/135). The clinical RR following the initial docetaxel regimen was 64%. The clinical responses to treatment with docetaxel followed by FEC according to response to initial docetaxel are shown in Table 3. Eight of the 135 patients (6%) progressed during docetaxel administration; 2 of 135 patients (1%) had disease progression during FEC. Of the 30 patients with triple-negative disease, 7 patients were observed to have disease progression following docetaxel treatment. One of the 17 patients with ER-positive, PgR-negative and HER2-negative tumors had disease

Table 2. Treatment-related adverse events

Adverse event, *n* (%)	DOC ($n = 135$)		FEC ($n = 29$)		Overall ($n = 35$)	
	All grades	Grade 3/4	All grades	Grade 3/4	All grades	Grade 3/4
Non-hematologic toxicities						
Infection with neutropenia	6 (4)	2 (1)	3 (2)	2 (2)	9 (7)	4 (3)
Fever	15 (11)	0	13 (10)	1 (1)	22 (16)	1 (1)
Infection (other)	3 (2)	1 (1)	2 (2)	0	4 (3)	1 (1)
Fatigue	82 (61)	0	84 (65)	2 (2)	98 (73)	2 (1)
Nausea	52 (39)	1 (1)	102 (79)	3 (2)	108 (80)	4 (3)
Vomiting	19 (14)	1 (1)	51 (40)	3 (2)	61 (45)	4 (3)
Anorexia	53 (39)	1 (1)	86 (67)	2 (2)	91 (67)	2 (1)
Stomatitis	50 (37)	1 (1)	51 (40)	0	68 (50)	1 (1)
Diarrhea	39 (29)	1 (1)	20 (16)	0	46 (34)	1 (1)
Phlebitis	2 (1)	1 (1)	2 (2)	0	4 (3)	1 (1)
Alanine aminotransferase	36 (27)	0	50 (39)	2 (2)	57 (42)	2 (1)
Aspartate aminotransferase	19 (14)	0	34 (26)	1 (1)	40 (30)	1 (1)
Nail changes	2 (1)	0	33 (26)	1 (1)	33 (24)	1 (1)
Weight loss	5 (4)	0	6 (5)	1 (1)	8 (6)	1 (1)
Creatinine	4 (3)	1 (1)	6 (5)	0	7 (5)	1 (1)
Edema	44 (33)	0	37 (29)	0	55 (41)	0
Hematologic toxicities						
Neutropenia	60 (44)	59 (44)	91 (71)	77 (60)	100 (74)	91 (67)
Leukopenia	69 (51)	50 (37)	101 (78)	66 (51)	108 (80)	76 (56)
Thrombocytopenia	13 (10)	0	28 (22)	2 (2)	31 (23)	1 (1)
Anemia	66 (49)	0	99 (77)	1 (1)	106 (79)	1 (1)
Febrile neutropenia	9 (7)	9 (7)	15 (12)	15 (12)	20 (15)	20 (15)

DOC, docetaxel; FEC, 5-fluorouracil, epirubicin and cyclophosphamide.

Table 3. Clinical response to DOC followed by FEC according to response to initial DOC treatment ($n = 135$)

Clinical response,[a] *n* (%)	Total[b]	Responder	Non-responder
Response to DOC			
Responder	87 (64)	79 (58)	8 (6)
Non-responder	48 (36)	27 (20)	21 (16)

[a]Overall response was confirmed after completion of chemotherapy in comparison with before docetaxel treatment.
[b]Percent value of each column was calculated by dividing by the total number of the evaluable patients ($n = 135$).

progression; while of the 53 patients with ER-positive, PgR-positive, and HER2-negative tumors and of the 9 patients with ER-positive, PgR-positive, and HER2-positive tumors, no patient had disease progression during docetaxel treatment. Among those with triple-negative disease, the majority of patients with disease progression after initial docetaxel were premenopausal [6/7 patients (86%)] and had solid-tubular carcinoma which characterized by solid cluster of cancer cells with expansive growth forming sharp borders [4/7 patients (57%)], as assessed using the Japanese Breast Cancer Society histological classification of breast tumors (21) (Table 4). Excluding the differences outlined above, there were no differences between patient and tumor characteristics for those with progressive disease versus non-progressive disease.

Twenty-seven of 48 non-responders to docetaxel (56%) had a response to FEC treatment; however, 8 of 87 responders to docetaxel (9%) showed no improvement in response with FEC treatment. Following chemotherapy, BCS was performed for 105 of 133 assessable patients (79%).

Pathological Response and Predictive Factors to Treatment

The primary endpoint—pCR rate—was 23% (95% CI, 16–31%). A near pCR rate of 6% (95% CI, 3–12%) resulted

Table 4. Clinical and pathologic characteristics of triple-negative breast cancer[a] for patients with progressive disease versus patients without progressive disease, following initial docetaxel therapy

Characteristic	Without PD	PD
No. of evaluable patients	23	7
Age, years		
Median	43	46
Range	(30–62)	(29–53)
Menopausal status, *n* (%)		
Premenopausal	15 (65)	6 (86)
Postmenopausal	8 (35)	1 (14)
Tumor stage		
T1	2 (9)	0
T2	14 (61)	5 (71)
T3	7 (30)	2 (29)
Nodal stage, *n* (%)		
N0	13 (57)	3 (43)
N1	10 (43)	4 (57)
Tumor type, *n* (%)		
Solid-tubular carcinoma	6 (26)	4 (57)
Papillotubular carcinoma	5 (22)	3 (43)
Scirrhous carcinoma	3 (13)	0
Unspecified invasive carcinoma	9 (39)	0

PD, progressive disease.
[a]Triple-negative tumors were defined as ER-negative, PgR-negative and HER2-negative primary breast cancer.

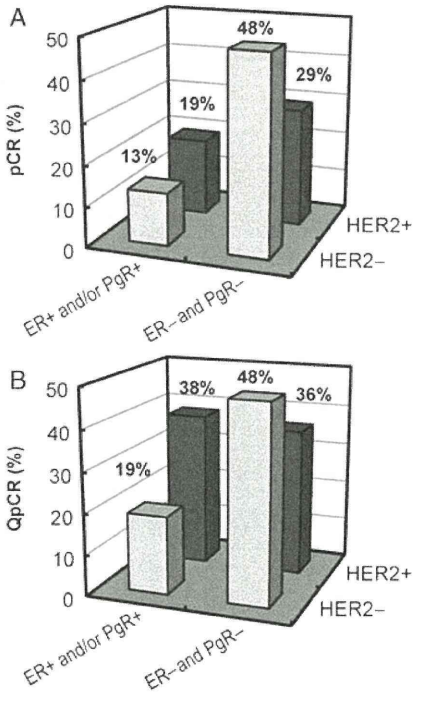

Figure 1. (A) Relationship between pCR versus HER2 and ER/PgR status following DOC and FEC ($n = 129$). (B) Relationship between QpCR versus HER2 and ER/PgR status following DOC and FEC ($n = 129$). Three patients were excluded from evaluable patients for pathologic response ($n = 132$) because of their unknown hormone receptor status. There were no near pCR case observed in triple-negative (ER−, PgR− and HER2−) diseases. DOC, docetaxel; ER, estrogen receptor; FEC, 5-fluorouracil, epirubicin, and cyclophosphamide; HER2, human epidermal growth factor receptor 2; pCR, pathologic complete response; PgR, progesterone receptor; QpCR, quasi-pathologic complete response.

in a QpCR rate of 29% (95% CI, 21–37%) when combined with the pCR. Pathological response of each subset population according to their hormone receptor and HER2 status is summarized in Fig. 1A and B. Patients with triple-negative disease had the highest pCR rate of 48% (95% CI, 29–68%). Near pCR was not observed in triple-negative disease. Patients with HER2-positive, ER-negative and PgR-negative tumors had a pCR rate of 29% (95% CI, 8–58%) and a QpCR rate of 36% (95% CI, 13–65%); patients with HER2-positive and ER-positive and/or PgR-positive tumors had a pCR rate of 19% (95% CI, 4–46%) and a QpCR rate of 38% (95% CI, 15–65%). Patients with HER2-negative and ER-positive and/or PgR-positive tumors had the lowest pCR and QpCR rates (13%; 95% CI, 6–23% and 19%; 95% CI, 10–30%, respectively). One of the seven patients who experienced clinical disease progression with initial docetaxel treatment had a QpCR following FEC.

The relationship between tumor pathological feature and pCR rate is shown in Table 5. The only variable found to be significantly associated with a pCR after docetaxel treatment was ER status.

Survival outcomes will be reported when the 5-year follow-up has been completed for this study.

DISCUSSION

This is the first report to evaluate the effectiveness of an initial docetaxel regimen for neoadjuvant therapy of Japanese patients with early-stage breast cancer. An additional component of the study was to analyze the data according to hormone receptor and HER2 status. Recently, Wildiers et al. (22) reviewed four adjuvant trials which had demonstrated the taxane-first regimens were favorable in terms of the relative drug dose intensity achieved. Also they mentioned larger non-randomized adjuvant studies for a series of 284 patients who first received three cycles of FEC followed by three cycles of docetaxel, the mean relative dose intensity was 91% for FEC and 76% for docetaxel, whereas in another series of 378 patients who received three cycles of docetaxel followed by four cycles of EC (epirubicin plus cyclophosphamide), a median docetaxel dose intensity of 100% was achieved. Therefore, they concluded such data suggest that the administration of a taxane first, followed by an anthracycline, may be preferable in line with the Norton–Simon hypothesis (23). In the JBCRG 01 study, the largest study to date to evaluate neoadjuvant chemotherapy in this patient population, the clinical and pathological responses

Table 5. Predictive variables for pCR before and following chemotherapy

Variables	Before treatment			After DOC			After FEC following DOC		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
Menopausal status: pre (versus post)	1.5	0.94–2.40	0.0923	1.52	0.94–2.47	0.0867	1.42	0.87–2.31	0.1575
Tumor size: ≥3 cm (versus <3 cm)	1.51	0.94–2.41	0.0881	1.45	0.90–2.34	0.1266	1.56	0.96–2.52	0.0724
ER: negative (versus positive)	0.58	0.32–1.03	0.0650	0.51	0.28–0.95	0.0331	0.58	0.32–1.05	0.0709
PgR: negative (versus positive)	0.66	0.34–1.28	0.2211	0.72	0.37–0.95	0.3408	0.65	0.33–1.27	0.2083
HER2: 3+ (versus <3+)	1.32	0.76–2.28	0.3251	1.41	0.80–2.47	0.2360	1.39	0.80–2.41	0.2445
Clinical response to DOC									
Response (versus no response)	—	—	—	0.64	0.38–1.07	0.0875	—	—	—
Clinical response to FEC following DOC									
Response (versus no response)	—	—	—	—	—	—	0.58	0.29–1.14	0.1160

CI, confidence interval; OR, odds ratio; pCR, pathologic complete response.

and safety of FEC followed by docetaxel were investigated (19). The eligibility criteria, treatment dose and distribution of patient characteristics (menopausal status, tumor stage, hormone receptor status and HER2 status) studied in the JBCRG 01 trial were similar to those investigated in the present JBCRG 03 study (19). The incidences of Grade 3/4 neutropenia and febrile neutropenia observed in the current study were similar to those reported in the JBCRG 01 trial (19). However, the rate of Grade 1/2 edema during docetaxel treatment was lower in the present study (33%) than in the JBCRG 01 study (41%), suggesting that docetaxel might be better tolerated when given up front than when administered after completion of prior chemotherapy. Further studies are warranted to assess quality of life and the incidence of edema in order to confirm the effect of administering docetaxel as the initial therapy.

Many different neoadjuvant chemotherapy schedules and dose regimens are used in clinical practice. The NSABP Protocol B-18 trial, which compared AC treatment before and after surgery, reported no difference in DFS between the two approaches (17). However, the rate of BCS was greater with neoadjuvant AC chemotherapy, and the prognosis of patients who obtained a pCR was also better with this treatment regimen (17). Several other regimens have been evaluated in an effort to increase the pCR rate. The addition of a taxane to an anthracycline-containing regimen has been shown to improve the pCR and clinical RRs (5,18). Furthermore, excellent results have been reported by the MD Anderson Cancer Center using a regimen of paclitaxel plus trastuzumab followed by FEC plus trastuzumab in patients with operable breast cancer and HER2 overexpression (24). However, few studies have evaluated initial taxane therapy followed by an anthracycline-containing regimen in this indication (24). Thus, it was decided to evaluate such a reverse regimen and to analyze the findings according to molecular subtypes. Importantly, the primary endpoint—pCR rate—achieved in the present study was 23% (95% CI, 16–31%), far exceeding our estimate of 12% (19). Even though the pCR rate here cannot be directly compared with the results from the JBCRG 01 trial (pCR rate: 12%, QpCR rate: 25%), the pCR rate from this study is a favorable result considering the similar patient characteristics in both trials (19).

The overall clinical RR of 79% was similar to that reported in the JBCRG 01 trial (74%) (19). Furthermore, the clinical RR following the initial docetaxel regimen was 64%, similar to the clinical response following the initial FEC regimen in the JBCRG 01 trial (61%) (19). The clinical RR following the initial docetaxel regimen, however, is lower in this study than those reported in other studies (71.7–85%) (25,26). It could be hypothesized that the clinical response might be influenced by the lower dose of docetaxel used in this study (75 mg/m^2) compared with the 100 mg/m^2 dose used in previous studies (25,26).

The rate of BCS observed in our study (79%) was similar to that reported in the JBCRG 01 trial (85%) (19). Unfortunately, the overall disease progression rate (5%) was not lowered by the use of docetaxel followed by FEC in this study, and was similar to that seen in the JBCRG 01 trial (6%) (19).

Although 7 of the 29 patients with triple-negative disease had disease progression during the initial docetaxel regimen, 14 of the 22 patients without disease progression (64%) achieved a QpCR. This QpCR rate is markedly higher compared with previous findings (27).

Our results indicate that if patients with triple-negative disease who experienced disease progression following initial docetaxel therapy were excluded, the pCR rate for this group of patients would have been higher. We thus compared the clinical and pathological characteristics between patients with triple-negative disease who experienced disease progression following the initial docetaxel regimen with those who did not have disease progression. However, no

significant differences in patient or tumor characteristics were seen between these patient groups. It was noted, however, that six of seven premenopausal patients (86%) and four of seven patients (57%) with solid-tubular carcinoma had disease progression following docetaxel therapy. Given the high incidence of disease progression among patients with triple-negative disease who had solid-tubular subtype tumors, this phenotype could be used in future studies to predict which patients are more likely to experience progressive disease following docetaxel therapy. Accordingly, the identification of patients with hormone receptor-positive and HER2-negative disease would also enable the selection of patients who are more likely to benefit from neoadjuvant chemotherapy. Thus, studying patients' molecular subtypes, and selecting appropriate chemotherapy regimens accordingly, has the potential to provide superior results to those of the JBCRG 03 trial.

Recently, it has been shown that basal-like breast cancer defined by five biomarkers [epidermal growth factor receptor (EGFR), cytokeratin 5/6 (CK5/6), ER, PgR and HER2 status] provides a more specific definition of basal-like breast cancer that predicts survival better than the triple-negative phenotype (27,28). In patients treated with anthracycline-based chemotherapy, tumors found to be positive for the basal markers corresponded to a cohort of patients with a significantly worse outcome (29). Thus in future trials, it may be beneficial to assess EGFR and CK5/6 status in patients with triple-negative disease to help predict patient survival.

Interestingly, the pCR rate (27%) following neoadjuvant chemotherapy in patients with HER2-negative breast cancer was higher in this study than in the JBCRG 01 study (14%), suggesting that this subpopulation may benefit from initial docetaxel treatment. Conversely, a lower QpCR rate was observed in HER2-positive patients (37%) in this study than in the JBCRG 01 trial (52.8%). This suggests that initial anthracyclines may be required for HER2-positive disease. A study by Buzdar et al. (24) reported that a high pCR rate of 60% was observed in patients with HER2-positive disease treated with the combination of paclitaxel plus trastuzumab followed by FEC plus trastuzumab, indicating that the HER2-positive population in the current study may have benefited further from concomitant trastuzumab therapy. These findings demonstrate the benefit of selecting the most effective chemotherapy regimen according to each patient's molecular subtype and initial response to neoadjuvant treatment.

One limitation of the study was that HER2-positive patients were not treated with trastuzumab, which has been shown to improve outcomes in patients with HER2-overexpressing breast cancer (24). Further studies investigating optimal treatment regimens for different molecular subtypes should include concurrent trastuzumab for patients with the HER2-positive phenotype.

In conclusion, docetaxel followed by FEC as neoadjuvant chemotherapy is a tolerable and effective regimen for patients with early-stage breast cancer. In addition, a high pCR rate made this regimen particularly promising in patients with triple-negative breast cancer. In the future, selection of a neoadjuvant chemotherapy regimen for operable breast cancer may be possible based on molecular subtype.

Acknowledgements

Editorial support was provided by sanofi-aventis. We wish to thank the patients who participated in the JBCRG 03 clinical trial. We also thank Drs Takashi Inamoto (Department of Breast Surgery, Kitano Hospital, Osaka, Japan), Akira Yamauchi (Department of Medicine for Cell Control, Faculty of Medicine, Kagawa University, Kagawa, Japan) and Hiroshi Ishiguro (Division of Clinical Trial Design and Management, Translational Research Center, Kyoto University, Kyoto, Japan) for their helpful advice.

Funding

This work was supported by Advanced Clinical Research Organization (http://www.npo-acro.jp/). Grant number 0520.

Conflict of interest statement

Dr Yasuo Ohashi received honorarium from Sanofi Aventis for lectures.

References

1. Kuerer HM, Newman LA, Smith TL, Ames FC, Hunt KK, Dhingra K, et al. Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. *J Clin Oncol* 1999;17:460–9.
2. Kaufmann M, Hortobagyi GN, Goldhirsch A, Scholl S, Makris A, Valagussa P, et al. Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: an update. *J Clin Oncol* 2006;24:1940–9.
3. Goldhirsch A, Glick JH, Gelber RD, Coates AS, Thurlimann B, Senn HJ. Meeting highlights: international expert consensus on the primary therapy of early breast cancer 2005. *Ann Oncol* 2005;16:1569–83.
4. Mauriac L, MacGrogan G, Avril A, Durand M, Floquet A, Debled M, et al. Neoadjuvant chemotherapy for operable breast carcinoma larger than 3 cm: a unicentre randomized trial with a 124-month median follow-up. Institut Bergonie Bordeaux Groupe Sein (IBBGS). *Ann Oncol* 1999;10:47–52.
5. Smith IC, Heys SD, Hutcheon AW, Miller ID, Payne S, Gilbert FJ, et al. Neoadjuvant chemotherapy in breast cancer: significantly enhanced response with docetaxel. *J Clin Oncol* 2002;20:1456–66.
6. Wolmark N, Wang J, Mamounas E, Bryant J, Fisher B. Preoperative chemotherapy in patients with operable breast cancer: nine-year results from National Surgical Adjuvant Breast and Bowel Project B-18. *J Natl Cancer Inst Monogr* 2001;30:96–102.
7. Bear HD, Anderson S, Smith RE, Geyer CE, Jr, Mamounas EP, Fisher B, et al. Sequential preoperative or postoperative docetaxel added to preoperative doxorubicin plus cyclophosphamide for operable breast cancer: National Surgical Adjuvant Breast and Bowel Project Protocol B-27. *J Clin Oncol* 2006;24:2019–27.

8. Morrow M, Krontiras H. Who should not receive chemotherapy? Data from American databases and trials. *J Natl Cancer Inst Monogr* 2001;30:109–13.
9. Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher ER, et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. *J Clin Oncol* 1998;16:2672–85.
10. Ferriere JP, Assier I, Cure H, Charrier S, Kwiatkowski F, Achard JL, et al. Primary chemotherapy in breast cancer: correlation between tumor response and patient outcome. *Am J Clin Oncol* 1998;21:117–20.
11. Machiavelli MR, Romero AO, Perez JE, Lacava JA, Dominguez ME, Rodriguez R, et al. Prognostic significance of pathological response of primary tumor and metastatic axillary lymph nodes after neoadjuvant chemotherapy for locally advanced breast carcinoma. *Cancer J Sci Am* 1998;4:125–31.
12. Buchholz TA, Hill BS, Tucker SL, Frye DK, Kuerer HM, Buzdar AU, et al. Factors predictive of outcome in patients with breast cancer refractory to neoadjuvant chemotherapy. *Cancer J* 2001;7:413–20.
13. Chang JC, Wooten EC, Tsimelzon A, Hilsenbeck SG, Gutierrez MC, Elledge R, et al. Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. *Lancet* 2003;362:362–9.
14. Chang JC, Wooten EC, Tsimelzon A, Hilsenbeck SG, Gutierrez MC, Tham YL, et al. Patterns of resistance and incomplete response to docetaxel by gene expression profiling in breast cancer patients. *J Clin Oncol* 2005;23:1169–77.
15. Ayers M, Symmans WF, Stec J, Damokosh AI, Clark E, Hess K, et al. Gene expression profiles predict complete pathologic response to neoadjuvant paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide chemotherapy in breast cancer. *J Clin Oncol* 2004;22:2284–93.
16. Ahr A, Karn T, Solbach C, Seiter T, Strebhardt K, Holtrich U, et al. Identification of high risk breast-cancer patients by gene expression profiling. *Lancet* 2002;359:131–2.
17. Fisher B, Brown A, Mamounas E, Wieand S, Robidoux A, Margolese RG, et al. Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-18. *J Clin Oncol* 1997;15:2483–93.
18. Bear HD, Anderson S, Brown A, Smith R, Mamounas EP, Fisher B, et al. The effect on tumor response of adding sequential preoperative docetaxel to preoperative doxorubicin and cyclophosphamide: preliminary results from National Surgical Adjuvant Breast and Bowel Project Protocol B-27. *J Clin Oncol* 2003;21:4165–74.
19. Toi M, Nakamura S, Kuroi K, Iwata H, Ohno S, Masuda N, et al. Phase II study of preoperative sequential FEC and docetaxel predicts of pathological response and disease free survival. *Breast Cancer Res Treat* 2008;110:531–9.
20. Japanese Breast Cancer Society. *General Rules for Clinical and Pathological Recording of Breast Cancer*. 15th edn. Tokyo, Japan: Kanehara Shuppan, 2004; 7.
21. Sakamoto G, Inaji H, Akiyama F, Haga S, Hiraoka M, Inai K, et al. General rules for clinical and pathological recording of breast cancer 2005. *Breast Cancer* 2005;12(Suppl):S1–27.
22. Wildiers H, Forceville K, Paridaens R, Joensuu H. Taxanes and anthracyclines in early breast cancer: which first?. *Lancet Oncol* 2010;11:219–20.
23. Simon R, Norton L. The Norton–Simon hypothesis: designing more effective and less toxic chemotherapeutic regimens. *Nat Clin Pract Oncol* 2006;3:406–7.
24. Buzdar AU, Valero V, Ibrahim NK, Francis D, Broglio KR, Theriault RL, et al. Neoadjuvant therapy with paclitaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide chemotherapy and concurrent trastuzumab in human epidermal growth factor receptor 2-positive operable breast cancer: an update of the initial randomized study population and data of additional patients treated with the same regimen. *Clin Cancer Res* 2007;13:228–33.
25. Gradishar WJ. Docetaxel as neoadjuvant chemotherapy in patients with stage III breast cancer. *Oncology (Williston Park)* 1997;11:15–8.
26. Amat S, Bougnoux P, Penault-Llorca F, Cure H, Charrier S, Praagh IV, et al. Primary chemotherapy for operable breast cancer: high pathological response rate induced by docetaxel. *Proc Am Soc Clin Oncol* 2000;19(Suppl):128a.[Abstract 500].
27. Liedtke C, Mazouni C, Hess KR, Andre F, Tordai A, Mejia JA, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. *J Clin Oncol* 2008;26:1275–81.
28. Schneider BP, Winer EP, Foulkes WD, Garber J, Perou CM, Richardson A, et al. Triple-negative breast cancer: risk factors to potential targets. *Clin Cancer Res* 2008;14:8010–8.
29. Cheang MC, Voduc D, Bajdik C, Leung S, McKinney S, Chia SK, et al. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. *Clin Cancer Res* 2008;14:1368–76.

Breast Cancer (2011) 18:299–308
DOI 10.1007/s12282-010-0212-y

ORIGINAL ARTICLE

Early metabolic response to neoadjuvant letrozole, measured by FDG PET/CT, is correlated with a decrease in the Ki67 labeling index in patients with hormone receptor-positive primary breast cancer: a pilot study

Shigeto Ueda · Hitoshi Tsuda · Toshiaki Saeki · Jiro Omata · Akihiko Osaki · Takashi Shigekawa · Jiro Ishida · Katsumi Tamura · Yoshiyuki Abe · Tomoyuki Moriya · Junji Yamamoto

Received: 22 February 2010 / Accepted: 19 May 2010 / Published online: 9 July 2010

Abstract
Purpose To assess whether the early metabolic response evaluated by ^{18}F-fluorodeoxy-glucose positron emission combined with computed tomography (FDG PET/CT) predicts the morphological, pathological, and cell-cycle responses to neoadjuvant endocrine therapy of hormone receptor-positive primary breast cancer.
Study design Eleven patients (12 tumors) with estrogen receptor-positive (Allred score 7 or 8) primary breast cancer were enrolled. All patients received a daily dose (2.5 mg) of letrozole for 12 weeks followed by surgery. Sequential FDG PET/CT scans were performed before treatment (baseline), at 4 weeks after the initiation of endocrine therapy (PET2), and prior to surgery (PET3). Tumors showing a 40% or more reduction and those showing a less than 40% reduction in the standardized uptake value maximum (SUV_{max}) at PET2 compared with the baseline PET were defined as metabolic responders and metabolic nonresponders, respectively. Change in tumor size as measured by ultrasound (morphological response), pathological response, and change in the Ki67 labeling index in tumor tissue (cell-cycle response) during the neoadjuvant letrozole therapy were compared between the metabolic responders and nonresponders.

S. Ueda · J. Omata · T. Moriya · J. Yamamoto
Department of Surgery, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan
e-mail: syueda2000@yahoo.co.jp

J. Omata
e-mail: dr20008@ndmc.ac.jp

T. Moriya
e-mail: tmoriya@ndmc.ac.jp

J. Yamamoto
e-mail: jyamamot@ndmc.ac.jp

T. Saeki · A. Osaki · T. Shigekawa
Breast Oncology Service, Tokorozawa Ichou Hospital, 1-7-25 Nishi-tokorozawa, Tokorozawa, Saitama, Japan
e-mail: tsaeki@saitama-med.ac.jp

A. Osaki
e-mail: aosaki@saitama-med.ac.jp

T. Shigekawa
e-mail: takshige@saitama-med.ac.jp

S. Ueda · T. Saeki · T. Shigekawa
Department of Breast Oncology, Saitama Medical University, International Medical Center, 1397-1 Yamane, Hidaka, Saitama 350-1298, Japan

A. Osaki
Department of Breast Oncology, Saitama Medical University, 38 Morohongo, Moroyama, Saitama 350-0495, Japan

J. Ishida · K. Tamura · Y. Abe
Tokorozawa PET Diagnostic Imaging Clinic, 7-5 Higashi-Sumiyoshi, Tokorozawa, Saitama 359-1124, Japan
e-mail: ishida@toko-pet.or.jp

K. Tamura
e-mail: tamurak@nn.iij4u.or.jp

Y. Abe
e-mail: abe@toko-pet.co.jp

H. Tsuda (✉)
Department of Basic Pathology, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan
e-mail: hstsuda@ncc.go.jp

S. Ueda
Headquarter Clinic, Mutual Aid Association, Ministry of Defense, 5-1 Honmura-chou, Ichigaya, Shinjuku-ku, Tokyo, Japan

Results The average decreases in SUV_{max} at PET2 compared with the baseline PET in the metabolic responders ($n = 6$) and the metabolic nonresponders ($n = 6$) were 60.9% (±21.3 SD) and 14.2% (±12.0 SD), respectively. At PET3 compared with the baseline PET, the metabolic responders showed a significantly higher decrease of 64.5% (±18.7 SD) ($p = 0.0004$), whereas the nonresponders showed a nonsignificant decrease of 16.7% (±14.1 SD) ($p = 0.06$). The morphological and pathological responses after letrozole therapy did not differ between the metabolic responders and nonresponders. The metabolic responders showed a marked decrease in the Ki67 labeling index at 2 weeks after the initiation of treatment (62.9%, ±35.9 SD, $p = 0.04$) and at surgery (91.7%, ±10.7 SD, $p = 0.03$) compared with the baseline values. In contrast, metabolic nonresponders showed no significant change in the Ki67 index either after 2 weeks of therapy or at surgery.

Conclusion Cell-cycle response monitored by the Ki67 labeling index correlates with metabolic response monitored by tumor SUV_{max}. Monitoring of tumor SUV_{max} using FDG PET/CT may be feasible to predict cell-cycle response to neoadjuvant endocrine therapy of primary breast cancer.

Keywords Breast cancer · FDG PET/CT · Neoadjuvant endocrine therapy · SUV

Introduction

Neoadjuvant endocrine therapy is a treatment option for patients with hormone receptor (HR)-positive (estrogen receptor-positive and/or progesterone receptor-positive) breast cancer, and the long-term efficacy of this approach is currently being studied [1, 2]. Some clinical trials have shown that neoadjuvant endocrine therapy can contribute to down-staging and to improved therapeutic efficacy in HR-positive breast cancers [3–5]. Therefore, early identification of patients who do or do not respond to endocrine therapy is essential, allowing patients with less endocrine-sensitive tumors to receive alternative treatments such as surgery or chemotherapy [6].

A randomized trial (Intermediate Marker Project: Anastrozole, Combination or Tamoxifen; IMPACT) compared the efficacy of neoadjuvant anastrozole versus neoadjuvant tamoxifen for 12 weeks in 330 postmenopausal women with HR-positive operable breast cancer [4]. The rate of breast-conserving surgery was significantly higher in the anastrozole group (45.7%) than in the tamoxifen group (22.2%) ($p = 0.03$). In an affiliated study of the IMPACT trial, the Ki67 labeling index as a proliferation biomarker was measured at the 2nd week and at the 12th week of endocrine therapy prior to surgery. The mean Ki67 labeling indices at the 2nd week and the 12th week were decreased further in the anastrozole group than in the tamoxifen group ($p = 0.004$) [7]. Furthermore, the decreases in Ki67 labeling indices after 2 and 12 weeks of endocrine therapy were significantly correlated with relapse-free survival of the patients after surgery [8]. These results suggest that early cell-cycle response to endocrine therapies as measured by the Ki67 labeling index is predictive of better clinical outcome in patients with HR-positive breast cancer [9]. In the P024 trial of neoadjuvant letrozole for 337 women with HR-positive breast cancer, the objective response rate on clinical palpation was significantly higher in the letrozole group (55%) than in the tamoxifen group (36%) after the treatment for 12 weeks prior to surgery ($p < 0.001$) [10].

Currently, ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET) for imaging is widely used as a non-invasive approach for the detection and staging of breast cancer [11]. FDG PET provides the standardized uptake value (SUV) of tumor glucose metabolism as a highly reproducible quantitative parameter [12, 13]. The response of tumors to chemotherapy can be quantified by measuring changes in tumor glucose metabolism using sequential FDG PET [14, 15]. Small-scale clinical trials have revealed that an early metabolic response of the tumor after one or two cycles of chemotherapy correlates with better clinical outcome of the patients [16–18].

By means of cDNA microarray and immunohistochemistry, we previously reported that expression levels of cell-cycle-related proteins (Ki67 and CENP-F) were increased in tumors showing high FDG uptake in comparison with tumors showing low FDG uptake, suggesting that the level of FDG uptake could be explained, at least in part by the early stage proliferative activity of breast cancer cells [19].

The aim of the present study was to evaluate the feasibility of FDG PET in combination with computed tomography (FDG PET/CT) monitoring as a modality to predict the effect of endocrine therapy in primary breast cancers. This pilot study of neoadjuvant setting was designed to assess the utility of FDG PET/CT for the prediction of response of node-negative and HR-positive breast cancers to letrozole in postmenopausal women, taking into account morphological and pathological responses, and the cell-cycle response measured by the Ki67 labeling index in the primary tumors.

Materials and methods

Patients

Eleven patients with HR-positive operable (T1-T2, N0, M0) breast cancer were prospectively recruited for this

study between December 2007 and March 2008. One patient had bilateral tumors, and thus a total of 12 tumors were assessed. To determine baseline staging of breast cancer, palpation, chest X-ray, ultrasound of the bilateral breast, axilla, and liver, as well as whole-body FDG PET/CT were performed. In all the 11 patients, no axillary involvement was detected by palpation, ultrasound, or FDG PET/CT.

Estrogen receptor (ER), progesterone receptor (PR), and c-erbB2 (HER2) were assessed immunohistochemically in specimens obtained by core needle biopsy. Inclusion criteria for this study were postmenopausal women having breast cancer with ER positivity (Allred score 4 or more) and/or PR positivity (Allred score 4 or more) and in whom chemotherapy was not indicated or who refused chemotherapy. Exclusion criteria were metastatic disease and severe diabetes mellitus.

Patients received a daily dose of letrozole (2.5 mg) for at least 12 weeks before surgery (Fig. 1). Core needle biopsy (CNB) was performed for all patients at baseline and at 2 weeks after initiation of endocrine therapy.

FDG PET/CT scans were performed before initiation of endocrine therapy (baseline PET), at 4 weeks after initiation of therapy (PET2), and prior to surgery (PET3). We considered FDG PET/CT scanning at 4 weeks after initiation of treatment was the best timing of early response assessment of SUV for the purpose of minimizing the effect of tissue repairing reaction to CNB. All patients underwent breast-conserving surgery or mastectomy at the Departments of Surgery of the National Defense Medical College (NDMC) Hospital or the Tokorozawa Icho Hospital, Tokorozawa, Japan.

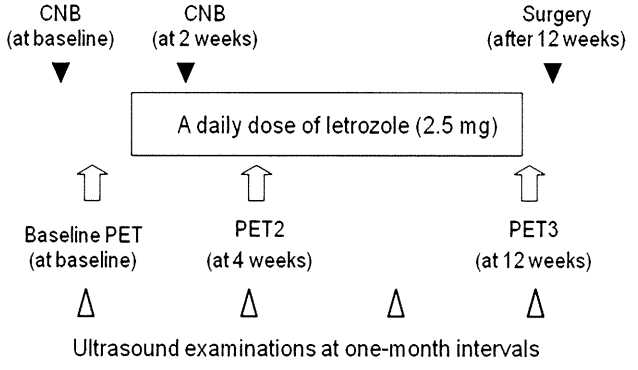

Fig. 1 Schematic presentation of the study design. The regimen of neoadjuvant endocrine therapy consisted of a daily dose (2.5 mg) of letrozole for 12 weeks. The treatment was monitored by serial FDG PET/CT at baseline (*baseline PET*), at 4 weeks (*PET2*) after the initiation of endocrine therapy, and at 12 weeks (*PET3*) after the initiation of endocrine therapy and prior to surgery. Ultrasound examination to measure tumor size was also performed at baseline and on a monthly basis. Evaluation of tumor size using ultrasound according to the RECIST guidelines [16] was performed by one experienced ultrasonographer to compare size reduction between baseline and the time at the completion of treatment. The intercurrent measurements of tumor size by ultrasound were for determination of progression; hence, discontinuation of the treatment was considered. Core needle biopsies for tumors were performed at baseline and at 2 weeks after the initiation of treatment. Surgery was performed after the completion of the endocrine therapy. The Ki67 labeling index of cancer cells was evaluated both in core needle biopsy specimens and in surgically resected specimens

This study was approved by the institutional review committee of the NDMC. Informed consent was obtained from each eligible patient.

FDG PET/CT and quantification of FDG uptake in primary tumors

The procedure for FDG PET/CT has been described previously [13]. Briefly, patients underwent ^{18}F-FDG PET/CT scans at Tokorozawa PET Imaging and Diagnostic Clinic (Tokorozawa, Japan). PET/CT imaging was performed with Biograph Duo (Siemens CTI). The Biograph Duo allows simultaneous collection of 64 slices over a span of 15.8 cm with a slice thickness of 2.5 mm and a transaxial resolution of 6.3 mm. All data were reconstructed with OSEM image. Patients fasted at least 6 h before PET/CT studies. One hour after intravenous administration of ^{18}F-FDG at about 3.7 MBq/kg, a transmission scan using CT for attenuation correction and anatomical imaging was acquired for 90 s. Blood glucose levels measured in each patient did not exceed 120 mg/dl.

A region of interest (ROI) was placed in the target lesions, including the highest uptake area (circle ROI, 10 mm in diameter), and SUV_{max} in the ROI was calculated. SUV_{max} is decay-corrected tissue activity divided by the injected dose per patient body. SUV_{max} was calculated using the following formula: SUV = activity in ROI (MBq/ml)/injected dose (MBq/kg body weight).

After the completion of FDG PET acquisition, the reconstructed attenuation corrected PET images, CT images, and combined images of matching pairs of the FDG PET and CT images were available for review in axial, coronal, and sagittal planes and in maximum intensity projections, three-dimensional cine-mode. At least two experienced nuclear medicine radiologists interpreted the FDG PET/CT images.

Criteria for morphological response on ultrasound imaging

Patients underwent ultrasound examination to measure tumor size during treatment. Evaluation of tumor size using ultrasound was performed twice by one experienced ultrasonographer to compare size reduction: at baseline and at the completion of treatment, according to the RECIST guidelines [16]. The longest diameter of a target lesion was

determined as the clinical tumor size. Intercurrent measurements of tumor size by ultrasound were performed only for the detection of patients with progressive disease.

The protocol stipulated that use of letrozole should be discontinued in patients who showed progressive disease (20% or more increase in size) by ultrasound and that such patients should be excluded from further participation in the study. However, no patients in this series had progressive disease.

Criteria for pathological response

Histological criteria for assessment of therapeutic response in breast cancer were taken from the General Rules for Clinical and Pathological Recording of Breast Cancer 2007 [20]. Pathological response was evaluated only from histological changes in the invasive area, and the presence of ductal components and/or lymph node metastasis was not evaluated. Pathologic complete response or pCR (Grade 3) was defined as the complete disappearance of invasive components of breast cancer in the pathologic specimen. The disappearance or marked degeneration of two-thirds or more of the tumor cells was defined as substantially effective (grade 2). The disappearance or marked degeneration of one-third to less than two-thirds was defined as moderately effective (grade 1b). The disappearance or marked degeneration of less than one-third of the tumor cells or mild tumor cell degeneration, regardless of the percentage, was defined as mildly effective (grade 1a). Almost no change in cancer cells after treatment was defined as not effective (grade 0). Pathological response was based on microscopic assessment of the surgical specimen by one breast pathologist (H.T.).

Pretherapeutic clinical and immunohistochemical parameters

Before the initiation of endocrine therapy, CNB was performed for diagnosis. Patient age, tumor size, histology, and the absence of clinically positive lymph nodes were recorded. Expressions of estrogen receptor (ER), progesterone receptor (PR), and c-erbB2 (HER2) were measured as described previously [21–23]. Scores for ER and PR were measured by the Allred scoring system [24].

Scoring of the Ki67 labeling index

Immunohistochemically, the Ki67 labeling index was measured to evaluate the proliferative activity of cancer cells. The Ki67 labeling index was counted for a minimum total of 300 cancer cells from 10 randomly selected high-power fields (400×) containing representative sections of the tumors. The value was calculated as the percentage of cells showing moderate to high staining intensity relative to the total number of cells [8, 9].

Statistical analysis

Statistical analysis was carried out with Statview 5.0 (SAS Institution Inc., Cary, NC). The Mann-Whitney U test and Student's t test were used to compare variables between metabolic responders and nonresponders. The Wilcoxon signed-ranks test was used to compare serial changes in Ki67 values between metabolic responders and metabolic non-responders. A simple regression analysis was used to determine the relationship between percentage reduction in SUV_{max} and percentage decrease in the Ki67 labeling index by a head-to-head comparison of each case. Differences at a p value of less than 5% were considered to be statistically significant.

Results

Patient characteristics

Table 1 shows the characteristics of 12 clinically node-negative tumors in 11 patients. The average patient age was 73.6 years [±9.3 standard deviation (SD)]. Eleven (92%) of the 12 tumors were invasive ductal carcinoma, and one (8%) was mucinous carcinoma. Average tumor size was 26.5 mm (±9.5 SD). Ten (83%) and 2 (17%) of the 12 tumors were ER-positive with Allred scores of 8 and 7, respectively. For PR status, four (33%) and two (17%) tumors had Allred scores of 8 and 7, respectively. In three (25%) tumors, the Allred score was 5 or 4, and in three tumors (25%) the Allred score was 0 or 1. Two (17%) of the 12 tumors exhibited HER2 overexpression (score 3+). There was no correlation of baseline SUV_{max} with PR or HER2 status.

Metabolic response by FDG PET/CT

Baseline PET and PET2 were performed for all 12 tumors. PET2 was performed on average 31.8 ± 4.3 days after initiation of endocrine therapy. PET3 was performed for 10 (83%) of the 12 tumors at 106.5 ± 16.3 days after initiation of treatment. The other two (17%) patients refused to undergo FDG PET/CT scanning at PET3 (Table 2).

Changes in SUV_{max} were compared among baseline PET, PET2, and PET3 (Fig. 2a). These results demonstrated the existence of a clear threshold at the point of PET2 between tumor no. 5 (31.4% reduction) and tumor no. 2 (43.4% reduction). We adopted 40% reduction in SUV_{max} as a tentative cutoff value between metabolic responders and metabolic nonresponders.

Table 1 Characteristics of the 12 clinically node-negative primary breast cancers in 11 patients

Serial number	Location	Age (year)	Tumor size (mm)	Histology	Allred score		HER2
					ER	PR	
1	Rt	80	36	Invasive ductal	7	0	3+
2	Lt	81	27	Mucinous	8	4	2+
3	Rt	78	33	Invasive ductal	8	1	3+
4	Lt	60	25	Invasive ductal	8	8	1+
5	Rt	77	27	Invasive ductal	8	5	1+
6	Lt	77	19	Invasive ductal	8	8	1+
7	Lt	82	49	Invasive ductal	7	7	2+
8	Rt	83	12	Invasive ductal	8	5	1+
9	Rt	70	23	Invasive ductal	8	8	2+
10	Lt	68	20	Invasive ductal	8	7	1+
11	Lt	70	22	Invasive ductal	8	0	0
12	Lt	75	24	Invasive ductal	8	8	0

Numbers 5 and 6 are bilateral tumors from a single patient

ER estrogen receptor, *PR* progesterone receptor, *Lt* left, *Rt* right

Table 2 SUV_{max} of tumors assessed by FDG PET/CT at baseline and at 4 and 12 weeks after initiation of neoadjuvant letrozole therapy, stratified by metabolic responders and metabolic nonresponders

Serial number	SUV_{max} by FDG PET/CT		
	Baseline	At 4 weeks	At 12 weeks
Responders			
2	3.48	1.97	1.64
6	6.01	3.13	2.44
8	1.88	0.91	ND
9	12.7	3.82	3.23
10	5.18	2.66	1.32
11	12.8	5.66	5.82
Average (±SD)	6.8 (4.8)	2.8 (1.9)	2.9 (2.0)
Nonresponders			
1	4.92	5.29	4.91
3	2.36	2.17	2.28
4	3.11	2.38	2.3
5	3.22	2.21	2.41
7	2	1.57	1.68
12	1.25	0.96	ND
Average (±SD)	2.8 (1.6)	2.4 (1.5)	2.7 (1.3)

ND not done, *SD* standard deviation

Metabolic responders and metabolic nonresponders comprised six (50%) tumors and six (50%) tumors, respectively. The average reduction in SUV_{max} at PET2 compared with the baseline PET was 60.9% (±21.3 SD) in metabolic responders ($p = 0.0009$) and 14.2% (±12.0 SD) in metabolic nonresponders ($p = 0.03$). In contrast, the average reduction in SUV_{max} at PET3 compared with the baseline PET was 64.5% (±18.7 SD) in metabolic responders ($p = 0.0004$) and 16.7% (±14.1 SD) in metabolic nonresponders ($p = 0.06$) (Fig. 2b). Figure 3 shows serial FDG PET/CT images of tumors in a metabolic responder (upper panels) and a metabolic nonresponder (lower panels).

Two patients having HER2-overexpressing tumors were included in the non-responding group. PR status was not correlated with metabolic response (data not shown).

Morphological response on ultrasound imaging

Tumors with a metabolic response showed an average 3.2-mm reduction in size, ranging from 0.5 to 5.9 mm ($p = 0.3$), whereas tumors with a metabolic nonresponse also showed an average 3.2-mm reduction in size, ranging from −4.4 to 10.4 mm ($p = 0.5$). There was no difference between the two groups (Fig. 4).

Pathological response

Pathologic response of the six metabolic responders fell into grade 1b in two (33%), grade 1a in three (50%), and grade 0 in one (17%). On the other hand, pathologic response of the six non-responders fell into grade 1a in four (67%) and grade 0 in two (33%). There were no tumors with an effect of grade 2 or 3. When tumors achieved grade 1b were classified as pathologic responder and tumors with grade 0 and grade 1a were classified as non-responders, two pathologic responders belonged to metabolic responders, although there was no statistical difference in pathologic response rate between metabolic responders and metabolic nonresponders ($p = 0.5$).

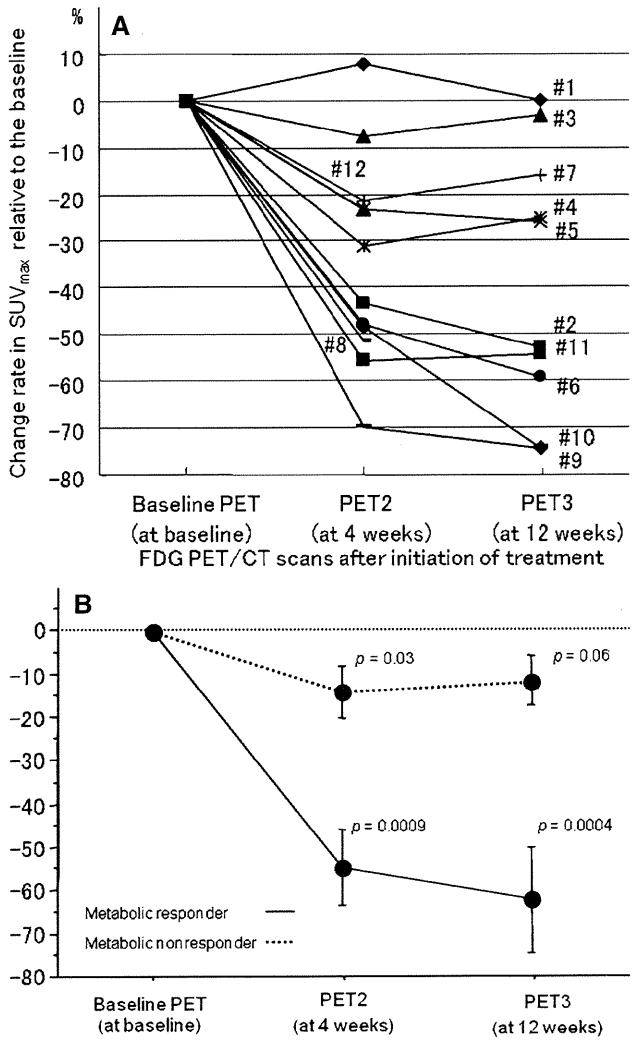

Fig. 2 Reduction in SUV_{max} of primary breast cancers measured by FDG PET/CT during neoadjuvant letrozole therapy. **a** Plots for all 12 tumors. In two tumors (nos. *8* and *12*), only baseline PET and PET2 were performed. A clear threshold at the points of PET2 appears to exist between tumor no. 5 (31.4% decrease) and tumor no. 2 (43.4% decrease). **b** Plots for average % reductions in SUV_{max} in metabolic responders and metabolic non-responders. Metabolic responders and metabolic nonresponders were tentatively defined as tumors with 40% or more reduction or with less than 40% reduction, respectively, in SUV_{max} at PET2 compared with SUV_{max} at baseline PET. The average reduction in SUV at PET2 compared with baseline PET was 60.9% [±21.3 standard deviation (SD)] in metabolic responders ($p = 0.0009$) and 14.2% (±12.0 SD) in metabolic nonresponders ($p = 0.03$). Likewise, the average reduction in SUV_{max} at PET3 compared with baseline PET was 64.5% (±18.7 SD) in metabolic responders ($p = 0.0004$) and 16.7% (±14.1 SD) in metabolic nonresponders ($p = 0.06$)

Cell cycle response measured by the Ki67 labeling index

Five of six metabolic responders showed a marked decrease in the Ki67 labeling index at 2 weeks and/or at surgery compared with the index at baseline (Fig. 5). In contrast, the degree of decrease in the Ki67 labeling index was relatively small or inverse in six metabolic nonresponders (Fig. 5). Compared with the baseline Ki67 labeling index, metabolic responders showed a significant decrease in the labeling index during therapy by Wilcoxon signed-ranks test: mean 62.9% (±35.9 SD) after 2 weeks of treatment ($Z = 2.0$, $p = 0.04$) and mean 91.7% (±10.7 SD) at surgery ($Z = 2.2$, $p = 0.03$). Conversely, metabolic nonresponders showed no significant decrease in labeling index by the Wilcoxon signed-ranks test: mean 9.8% (±26.3 SD) after 2 weeks of treatment ($Z = 0.8$, $p = 0.4$) and mean −49.5% (±138.8 SD) at surgery ($Z = 0.5$, $p = 0.6$) (Fig. 5). That analysis supported the validity of 40% SUV_{max} reduction of the cutoff value between the metabolic responders and the metabolic nonresponders.

Discussion

This study revealed that FDG PET/CT measurements were correlated with cell-cycle response to neoadjuvant letrozole in HR-positive breast cancers. When the 12 tumors were divided into metabolic responders and nonresponders according to a tentative cutoff value of 40% reduction in SUV_{max} at PET2 compared with the baseline PET, the former showed a further decline of SUV_{max} at PET3 ($p = 0.0004$), whereas the latter showed no significant change of SUV_{max} at PET3 ($p = 0.06$). On the contrary, the assessment of morphological response did not reveal significant differences between pre-therapeutic and post-therapeutic tumor sizes in both metabolic responders ($p = 0.3$) and nonresponders ($p = 0.5$).

Previous studies have concluded that 3 or 4 months of treatment with neoadjuvant letrozole for postmenopausal patients with breast cancer provides incremental clinical benefit for these patients. However, these studies did not determine the optimum duration of treatment. Therefore, Krainick-Strobel et al. [25] investigated the optimal duration of neoadjuvant letrozole therapy in postmenopausal patients with HR-positive breast cancer. Their data suggested that prolonged treatment for up to 8 months could result in greater tumor shrinkage compared with treatment for 4 months. From the present observations of metabolic response as well as a decrease in the Ki67 labeling index after the initiation of endocrine therapy, we suggest prolonged treatment duration of neoadjuvant letrozole therapy for 3 months or longer might be effective, especially in metabolic responders.

In recent studies of neoadjuvant chemotherapy, early metabolic response monitored by serial FDG PET scans after one or two cycles of chemotherapy was correlated with the pathological response of primary breast cancer [17, 18, 26–28]. With regard to pathological response in

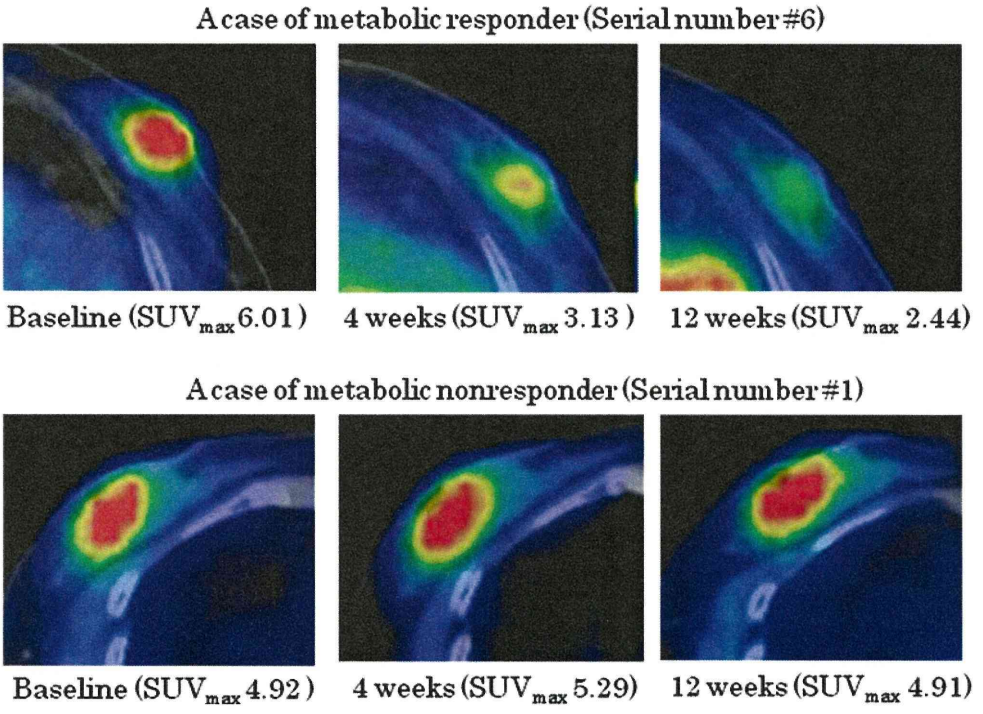

Fig. 3 Transversal slice images from FDG PET/CT of breast lesions before neoadjuvant letrozole therapy (*left*), at 4 weeks after the initiation of therapy (*middle*), and after 12 weeks of therapy and prior to surgery (*right*). A tumor classed as a metabolic responder (serial no. 6; *upper row*) shows SUV_{max} values of 6.01 (*left*), 3.13 (*middle*), and 2.44 (*right*). A tumor classed as a metabolic nonresponder (serial no. 1; *lower row*) shows SUV_{max} values of 4.92 (*left*), 5.29 (*middle*), and 4.91 (*right*)

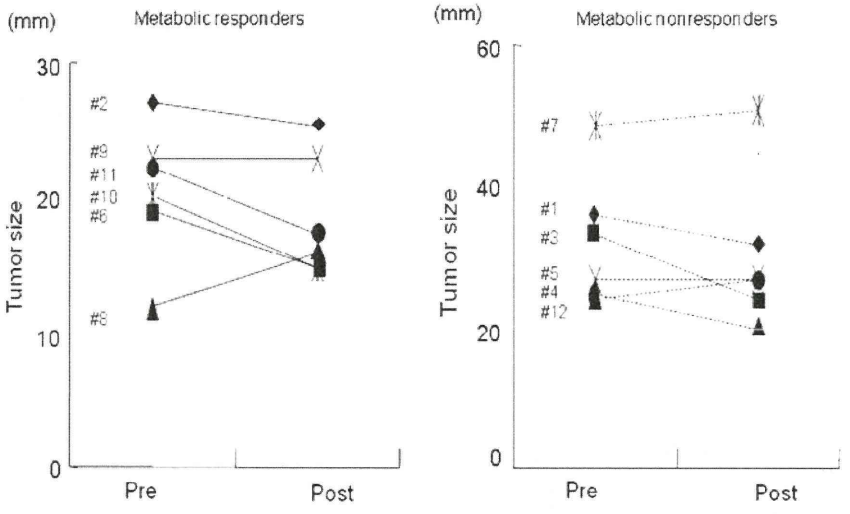

Fig. 4 Box plot of tumor size before and after neoadjuvant letrozole therapy of hormone-receptor-positive primary breast cancers. Among the six tumors with a metabolic response, four (67%) showed a decrease in tumor size, whereas two (33%) showed no change or an increase in tumor size. Among the six tumors with a metabolic nonresponse, three (50%) showed a decrease in tumor size, whereas the other three (50%) showed an increase in tumor size. Tumors with a metabolic response showed an average 3.2-mm reduction in size, ranging from 0.5 to 5.9 mm ($p = 0.3$), and tumors with a metabolic nonresponse also showed an average 3.2-mm reduction in size, ranging from −4.4 to 10.4 mm ($p = 0.5$). Each serial number in the box plot corresponds to a tumor

neoadjuvant endocrine therapy, it was shown that histological criteria for evaluation of therapeutic effect remained good not only for neoadjuvant chemotherapy, but also for neoadjuvant endocrine therapy [29]. In the present study, neither tumors of metabolic responders nor those of metabolic nonresponders achieved remarkable changes of grade 2 or 3 after surgery. However, the therapeutic effect of grade 1b was observed only in metabolic responders, and, if a larger number of cases were studied, metabolic response may be able to predict pathological response to neoadjuvant endocrine therapy.

Cell-cycle responses or decreases in the Ki67 labeling index both after 2 weeks of endocrine therapy and at surgery in comparison with the labeling index at the baseline were significantly greater in metabolic responders than in metabolic nonresponders. These data suggest that the

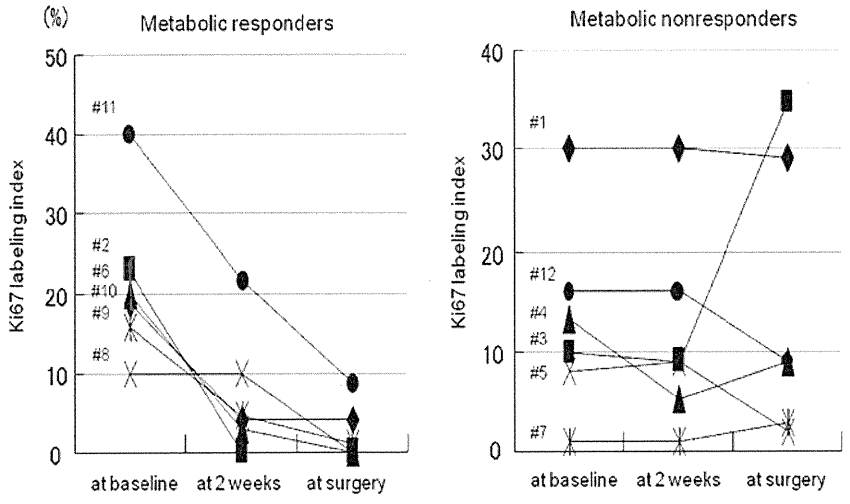

Fig. 5 Box plot of the Ki67 labeling index in primary breast cancers at baseline, at 2 weeks after initiation of neoadjuvant letrozole therapy, and at surgery. Five of six metabolic responders showed a marked decrease in the Ki67 labeling index at 2 weeks and/or at surgery compared with the index at baseline. In contrast, the degree of decrease in the Ki67 labeling index was relatively small or inverse in six metabolic nonresponders. Metabolic responders showed an average 62.9% (±35.9% SD) decrease in the Ki67 labeling index at 2 weeks after initiation of therapy ($z = 2.0$, $p = 0.04$) and an average 91.7% (±10.7% SD) decrease at surgery ($z = 2.2$, $p = 0.03$), compared with baseline. Metabolic nonresponders showed no significant change in the Ki67 labeling index at 2 weeks after initiation of therapy ($z = 0.8$, $p = 0.4$) or at surgery ($z = 0.5$, $p = 0.6$) compared with baseline. These analyses were performed by Wilcoxon signed-ranks test. Each serial number corresponds to a tumor

metabolic response of tumors to endocrine therapy might be underpinned by this marked suppression of proliferative activity in the 2 weeks after initiation of treatment.

Dowsett et al. [8] compared neoadjuvant endocrine therapy using anastrozole or tamoxifen or the combination of anastrozole plus tamoxifen in 158 patients with hormone receptor-positive primary disease and reported that the higher Ki67 labeling index after 2 weeks of endocrine therapy was significantly associated with shorter recurrence-free survival. However, measurement of the tumor Ki67 labeling index has limitations in that tissue samples obtained by means of CNB might be too small for proper assessment, since Ki67 expression in tumors may not be evenly distributed [3, 6]. Because tumor SUV_{max} values closely correlate with proliferative activity, FDG PET appears to be a less invasive and more easily applicable method for assessing response than measurement of the Ki67 labeling index in CNB samples.

Currently, a prospective clinical trial of neoadjuvant endocrine therapy using FDG PET and 16 alpha ^{18}F-fluoroestradiol (FES) PET, which uses a radio-labeled estradiol tracer as a marker for hormone sensitivity of breast cancer, to assess response is ongoing (see ClinicalTrials.gov, http://clinicaltrials.gov). The trial (NCT00362973) will investigate whether FDG PET and FES PET are useful for evaluating early response to treatment in patients receiving hormone therapy or trastuzumab for HR-positive primary breast cancer. Patients will undergo PET scans at baseline and at 2 weeks after the initiation of neoadjuvant endocrine therapy. CNB is also performed after 2 weeks of therapy to monitor tumor proliferation by the Ki67 index. The trial will assess the predictive value of FDG PET and/or FES PET for determining endocrine sensitivity in patients with HR-positive breast cancer.

This study has certain limitations. The chief weakness is a small number of patients. A much larger study with long-term follow-up would be necessary to determine directly whether FDG PET/CT has any role to play in the early prediction of hormone responsiveness. Second, we failed in a simultaneous comparison between Ki67 levels of the biopsy and SUV measured by PET at 2 weeks after the initiation of treatment because of patient availability. Third, we were only able to set tentatively the optimal cutoff of SUV_{max} change for differentiating responders and non-responders due to the lack of comparison data between metabolic response and clinicopathologic outcomes.

Recently, some investigators proposed the concept of a "cell cycle response" as a post-treatment endpoint based on the Ki67 value with 1% or less in the infiltrating component of the tumor that might have properties similar to the pathological complete response used as an endpoint in trials of neoadjuvant chemotherapy [30, 31]. Therefore, it is of note that when tumors with 1% or less of posttherapeutic Ki67 value are considered as having a favorable outcome, receiver operating characteristic (ROC) curve analysis provided a 43.4% decrease in SUV_{max} as the optimized cutoff value with sensitivity of 100% and specificity of 87.5%. This cutoff value was almost