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ORIGINAL ARTICLE

A phase II study of metronomic paclitaxel/cyclophosphamide/capecitabine followed by 5-fluorouracil/epirubicin/cyclophosphamide as preoperative chemotherapy for triple-negative or low hormone receptor expressing/HER2-negative primary breast cancer

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Abstract

Purpose Better treatments for triple-negative breast cancer (TNBC) are needed. To address this need, we studied the effects of preoperative metronomic paclitaxel/cyclophosphamide/capecitabine (mPCX) followed by 5-fluorouracil (FU)/epirubicin/cyclophosphamide (FEC) as preoperative chemotherapy in TNBC patients.

Methods Forty primary TNBC patients received four cycles of metronomic paclitaxel (80 mg/m^2 on Days 1, 8, and 15), cyclophosphamide (50 mg/body daily), and capecitabine (1,200 mg/m^2 daily), followed by four cycles of 5-FU (500 mg/m^2), epirubicin (100 mg/m^2), and cyclophosphamide (500 mg/m^2) every 3 weeks. The primary end point was the pathological complete response (pCR) rate.

Results Forty patients formed the intent-to-treat population. The median dose intensities of paclitaxel, cyclophosphamide, and capecitabine were 89.7, 92.1, and 89.8 %, respectively. Five patients discontinued mPCX and two discontinued FEC, primarily because of adverse events, resulting in a per-protocol population (PPS) of 33 patients. The pCR (ypT0/Tis ypN0) rate was 47.5 % (19/40) in the intent-to-treat population and 54.5 % (18/33) in the PPS. The clinical response rates were 36/40 (90.0 %) and 31/33 (93.9 %) in the intent-to-treat and PPS, respectively. The breast conservation rate was 72.7 % (24/33), and 5/13 patients underwent partial resection instead of pre-planned total mastectomy. Grade 3–4 adverse events included neutropenia (35 %), leukopenia (25 %), and hand-foot syndrome (8 %).

Conclusions Metronomic PCX followed by FEC chemotherapy was associated with a high pCR rate and low toxicity in TNBC patients. Further studies of this regimen in larger numbers of patients are warranted.

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Keywords Triple-negative breast cancer · Metronomic chemotherapy · Weekly paclitaxel · Oral cyclophosphamide · Capecitabine · pCR

Introduction

Triple-negative breast cancer (TNBC) accounts for ~15 % of all breast cancers [1–4]. The prognosis of TNBC is generally favorable in patients with pathological complete response (pCR), but is quite poor in patients with residual invasive tumors [5]. pCR rates in TNBC vary among chemotherapy regimens, adding to the challenge of treating TNBC. For example, anthracycline-based chemotherapy has a pCR rate of 17–27 % [5–8], increasing to 45 % following the addition of a taxane [9]. Sequential therapy with paclitaxel and anthracyclines has achieved a pCR rate of 28 % in TNBC [5].

A typical sequential therapy for TNBC comprises weekly paclitaxel followed by FEC. However, the outcomes of this regimen are unsatisfactory for TNBC, and more effective therapeutic options are needed [10].

The combination of capecitabine and cyclophosphamide appears to be promising, with an all-oral combination giving a response rate of >40 % in metastatic breast cancer, and is both feasible and well tolerated [11, 12]. The addition of capecitabine to a taxane achieved greater efficacy than a taxane/anthracycline combination [13], possibly because of the synergistic effect of increasing PyNPase activity [14, 15]. A combination of low-dose capecitabine and weekly paclitaxel yielded an overall response rate of 46.5 % in metastatic breast cancer [16]. A paclitaxel/cyclophosphamide combination was effective in patients with advanced recurrent breast cancer [17]. Dellapasqua et al. [18] reported that low-dose daily metronomic oral capecitabine and cyclophosphamide (mXC) combined with bevacizumab was effective for treating advanced breast cancer and minimally toxic. These results led to the hypothesis that a combination of paclitaxel, cyclophosphamide, and capecitabine (PCX) is feasible for chemotherapy in breast cancer patients. Two studies [19, 20] have demonstrated that the efficacy and tolerability of paclitaxel administered every week were better than those of paclitaxel administered every 3 weeks. In the first of these reports, the Cancer and Leukemia Group B (CALGB) trial revealed that weekly paclitaxel was associated with a greater response rate, together with a longer time to progression and longer overall survival than administration every 3 weeks. In that study, neutropenia was more common in patients treated every 3 weeks, while neuropathy was more common in patients treated every week. The meta-analysis conducted in the second report [20] confirmed that administration of paclitaxel every week conferred a survival benefit compared with administration every 3 weeks. Weekly paclitaxel was therefore comparable to the concept of metronomic therapy. In the present study, therefore, the promising regimen of the three drugs is referred to as metronomic paclitaxel, cyclophosphamide, and capecitabine (mPCX) [metronomic paclitaxel (80 mg/m^2 on Days 1, 8, and 15), cyclophosphamide (50 mg/body daily), and capecitabine (1,200 mg/m^2 daily), followed by four cycles of 5-FU (500 mg/m^2), epirubicin (100 mg/m^2), and cyclophosphamide (500 mg/m^2) every 3 weeks] and was based on the mCX regimens described above.

Metronomic chemotherapy regimens using combinations of standard drugs that are widely used to treat breast cancer are now being implemented in clinical trials in cancer patients and are proving as effective as maximum therapeutic dose chemotherapeutic regimens but with less toxicity [21–25]. The lower toxicity of these regimens is the main rationale for their adoption [26]. In this study, we examined the histologic effects and safety of four cycles of neoadjuvant mPCX followed by four cycles of neoadjuvant 5-fluorouracil (5-FU)/epirubicin/cyclophosphamide (FEC) in patients with TNBC. Outcomes included tumor response, rate of breast-conserving surgery, and toxicity.

Methods

Patients

Females aged 20–70 years with primary TNBC or HER2-negative breast cancer with low estrogen receptor/progesterone receptor expression (<10 %) were eligible for this study. Exclusion criteria included chemotherapy or hormone therapy for breast cancer in the last 5 years; active double cancer; synchronous bilateral breast cancer; male breast cancer; infection or suspected infection; serious heart disease or history thereof; poorly controlled diabetes mellitus; gastrointestinal ulcer or hemorrhage; or any serious comorbidities or a history of drug allergies that may interfere with treatment. Written informed consent was required to participate in the study. The study protocol was approved by the institutional ethics committees at all study locations, and the procedures followed were in accordance with the ethical standards of these committees and the Helsinki Declaration. This trial was registered on the University Hospital Medical Information Network (identifier: UMIN000003570).

Study design and treatment

This was a multicenter open-label study. Eligible patients received four cycles of mPCX followed by four cycles of FEC (Fig. 1a). Each cycle consisted of 3 weeks with a time

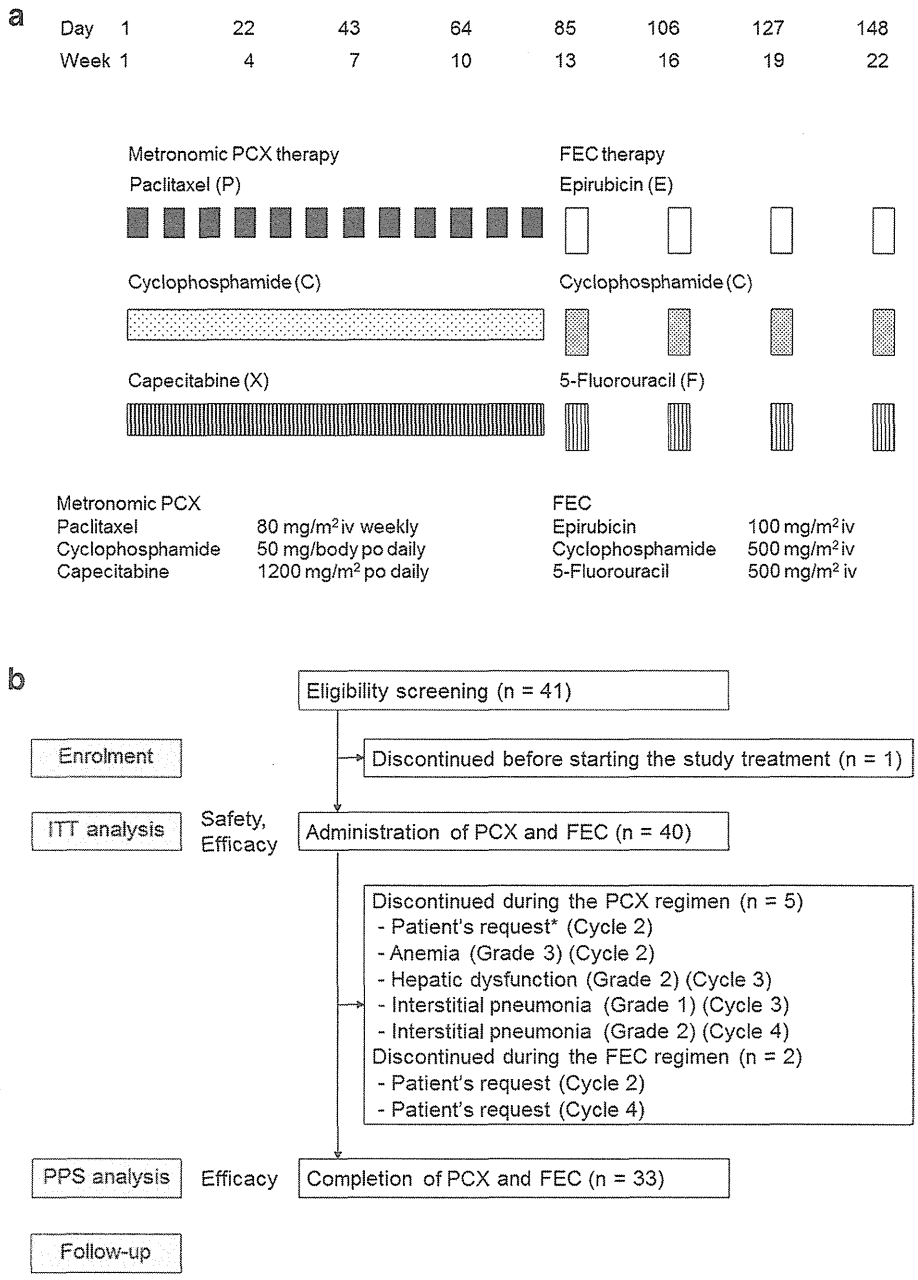

Fig. 1 **a** Study design. During metronomic PCX therapy, patients received intravenous paclitaxel (80 mg/m^2) on Days 1, 8, and 15, oral cyclophosphamide (50 mg) on Days 1–21, and oral capecitabine (1,200 mg/m^2) on Days 1–21 every 3 weeks for four cycles. Patients then received intravenous 5-FU (500 mg/m^2) on Day 1, intravenous epirubicin (100 mg/m^2) on Day 1, and intravenous cyclophosphamide (500 mg/m^2) on Day 1, every 3 weeks for four cycles. **b** Patient disposition. * P-FEC was administered after discontinuation

window of ±3 days, and surgery was performed 3–8 weeks after the last day of the fourth FEC cycle.

The primary efficacy end point was pCR rate. Secondary end points were the rates of breast-conserving surgery and safety findings.

Enrollment started in March 2010. A key concern of the study was to ensure the safety and tolerability of the new regimen. To that end, in March 2011, the efficacy and safety evaluation committee did an interim evaluation on 10 patients initially enrolled, confirming the efficacy and safety of the treatment. The committee members then unanimously voted for continuation of the study based on the results of the interim evaluation.

Assessments

The primary lesion and metastases to lymph nodes were measured within 1 month before treatment initiation, after the second cycle of metronomic mPCX, and after the fourth cycles of mPCX and FEC. The primary end point of pCR was defined as ypT0/Tis and ypN0, namely an absence of invasive cancer in the breast and lymph nodes. Surgical specimens were sectioned into 5-mm-thick slices, and all cut surfaces were examined. pCR in the primary lesion was categorized, as outlined by Kuroi et al. [27] as strict pCR (spCR), pCR with in situ carcinoma (pCRinv), comprehensive pCR (CpCR), near pCR (response very close to that of

SpCR, but with a small number of cancer cells), and quasi-pCR (includes CpCR and near pCR). In the axillary lymph nodes, ITC pN0(i+) of ≤0.2 mm was classified as pN0. A pathologic non-responder was defined as having invasive cancer on pathologic examination (pINV). Adverse events were assessed based on the common terminology criteria for adverse events (CTCAE) version 4.0, except for "nail changes," which were evaluated using the CTCAE version 3.0.

Treatment protocol

The rationales for the choice of paclitaxel [28–35], cyclophosphamide [36], and capecitabine [14–16, 18, 36–39] for metronomic PCX (mPCX) therapy and the appropriate doses [11, 17, 38–41] were determined on the basis of previous clinical studies. Weekly administration of paclitaxel at a dose of 80 mg/m^2 is a standard regimen for breast cancer and was therefore used in this study. For capecitabine, when we converted the doses used in combination regimens [11–13, 16, 18] to daily doses, we found that the dose ranged from 1,000 to 1,670 mg/m^2/day. We decided to use a dose close to the middle of this range of 1,200 mg/m^2 daily (administered as 600 mg/m^2 twice daily). For cyclophosphamide, a daily dose of 50 mg is thought to be safe and effective. Accordingly, the metronomic chemotherapy cycle consisted of paclitaxel (80 mg/m^2 on Days 1, 8, and 15), cyclophosphamide (50 mg once daily), and capecitabine (600 mg/m^2 twice daily) every 3 weeks for 12 weeks.

Metronomic chemotherapy involves administering cytotoxic antineoplastic agents at a low dose, avoiding dose-limiting toxicities by exposing endothelial cells, which proliferate slowly, to continuous low doses of cytotoxic antineoplastic agents.

Because mPCX is a new concept, the criteria for dose reduction and interruption were clearly defined to reduce the incidence of adverse events.

mPCX or the relevant component drug was to be suspended or discontinued in the case of neutrophil count ≤1,000/mm^3 (Grade 3), hand-and-foot syndrome (Grade 2–3), peripheral neuropathy, arthralgia or myalgia (Grade 3) or cystitis (Grade ≥2).

FEC therapy was to be discontinued in the case of Grade 3 neutropenia with fever of ≥38 °C; platelet count <25,000/mm^3 or hemorrhage/platelet transfusion with decreased platelet count; non-hematologic toxicities of ≥Grade 3 (except nausea, vomiting, and anorexia).

The overall safety of the study protocol was assessed after 10 subjects had been enrolled. If >2 subjects were unable to start the second cycle of mPCX therapy within 3 weeks of completing the first because the criteria for starting mPCX or paclitaxel (Online Resource 1) were not met, discontinuation of the study was to be considered.

Sample size and statistical analyses

The sample size was calculated based on a pCR rate of 28 % with preoperative paclitaxel FAC (5-FU/doxorubicin/cyclophosphamide)/FEC therapy and 20 % with an anthracycline-based regimen (FAC/FEC/AC) [5]. Because cyclophosphamide and capecitabine were coadministered with paclitaxel, the expected pCR rate was 40 % at a threshold of 20 %. Under those conditions with $\alpha = 0.05$ and $\beta = 0.2$, 36 evaluable subjects were required, and a sample size of 40 patients was considered sufficient to allow for discontinuations. The proportions of patients with a partial response (PR) or better and of those with a complete response (CR; objective CR), together with 95 % confidence intervals, were calculated from the distribution of the objective response.

Histologic response was measured as the proportion of patients with pCR. For adverse events, the proportions of Grade 1–4 events were calculated. All analyses are presented descriptively as the n (%) of patients or median (range).

Results

Patient characteristics

Forty-one patients were enrolled into the study and 40 (median age 52 years; range 33–69 years) underwent at least one cycle of treatment and were included in the intent-to-treat (ITT) population (Fig. 1b). The median tumor size was 23.7 mm (range 3.5–82 mm) and was classified as N(+) in 40 % (16/40) and weakly positive for ER (i.e., 1–9 %) in 17.5 % (7/40) of patients (Table 1). Five patients withdrew during mPCX at the patient's request in two cases and because of adverse events in three. Two patients withdrew during FEC at their own request. Therefore, the per-protocol population (PPS) consisted of 33 patients (Fig. 1b).

Treatment exposure

Because this regimen was being evaluated for the first time, tolerability was assessed in terms of the relative dose intensity (RDI) of each component, which was classified as RDI in all patient groups (Online Resource 2). The RDI was high in the mPCX phase (paclitaxel 89.7 %; cyclophosphamide 92.1 %; capecitabine 89.8 %) and in the FEC phase (epirubicin 89.8 %).

Table 1 Patient characteristics

Characteristic	Value
N	40
Age, years	
Median (range)	52.0 (33.0, 69.0)
PS	
0	37 (92.5 %)
1	3 (7.5 %)
Tumor stage status	
T1	8 (20.0 %)
T2	27 (67.5 %)
T3	5 (12.5 %)
Tumor size, mm[a]	
Median (range) (mm)	23.7 (3.5, 82.0)
Node status	
N0	24 (60.0 %)
N1	16 (40.0 %)
Disease stage	
Stage I	5 (12.5 %)
Stage IIa	21 (52.5 %)
Stage IIb	10 (25.0 %)
Stage IIIa	4 (10.0 %)
Menopausal status	
Premenopausal	19 (47.5 %)
Postmenopausal	21 (52.5 %)
ER (IHC)	
0 %	33 (82.5 %)
1–9 %	7 (17.5 %)
PgR (IHC)	
0 %	39 (97.5 %)
1–9 %	1 (2.5 %)
HER2 (IHC)	
0	28 (70.0 %)
1	8 (20.0 %)
2	1 (2.5 %)
NA	3 (7.5 %)
Histological grade (B&R classification)	
1	5 (12.5 %)
2	11 (27.5 %)
3	23 (57.5 %)
Unknown	1 (2.5 %)
Sentinel node lymph biopsy before starting the study treatment	
No	36 (90.0 %)
Yes	4 (10.0 %)
n0	4 (100.0 %)
n+	0 (0.0 %)
Surgical treatment planned before starting the study treatment	
BCS	25 (62.5 %)
Mastectomy	15 (37.5 %)

PS performance status, *ER* estrogen receptor, *PgR* progesterone receptor, *HER2* human epidermal growth factor receptor, *IHC* immunohistochemistry, *B&R* Bloom and Richardson grading system, *BCS* breast-conserving surgery

[a] Measured by magnetic resonance imaging or computed tomography; if both were available, the magnetic resonance imaging-determined size was used

Clinical response

The clinical response rate was calculated following an objective evaluation of the clinical response based on palpation and MRI/CT (Table 2). The clinical response rate (based on MRI/CT) was 90.0 % (36/40) in the ITT population and 93.9 % (31/33) in the PPS.

pCR

pCR was achieved after mPCX and FEC by 47.5 % (19/40) of patients in the ITT population and in 54.5 % (18/33) of patients in the PPS. The results for all grades of pCR in both populations are shown in Table 3.

Further analysis on weakly ER-positive patients ($n = 7$) revealed that invasive breast cancer disappeared in four patients (ypT0/Tis) after mPCX-EFC therapy, three of whom were axillary lymph node negative (ypN0). The specific results in these seven patients were SpCR *n*0 ($n = 1$), SpCR *n*(+) (1), pCRinv n0 (2), near pCR *n*(+) (2), and non-pCR *n*0 (1).

Relationship between tumor response rates after mPCX and pathological response

Figure 2 shows the tumor response rates in individual patients. Eleven of the 33 patients in the PPS achieved a clinical CR (i.e., a decrease in lesion size of 100 % on MRI/CT). Ten of these 11 patients achieved a pCR (ypT0/Tis ypN0). For the remaining patient, the histological rating for the primary focus was SpCR (no residual invasive or non-invasive tumor); however, this patient was positive for lymph node invasion.

Surgical procedures

Breast-conserving surgery was planned in 25 patients in the ITT population (including 20 in the PPS), and the breast was successfully conserved after surgery in 23 patients (19 from the PPS), corresponding to a success rate of 92.0 (95 % CI: 81.4–100.0 %; 95 % success rate and 95 % CI: 85.4–104.6 % in the PPS). The other two patients underwent total mastectomy.

In 15 patients in the ITT population (including 13 in the PPS), total mastectomy was planned, but breast-conserving surgery (BCS) was possible in six patients (40 %, 95 % CI: 15.2–64.8 %; BCS was possible in five (38.5 %) of the 13 patients in the PPS (95 % CI: 12.0–64.9 %)). The other nine patients (eight from the PPS) underwent total mastectomy, as planned. Therefore, the overall rate of breast conservation in the ITT was 72.5 % (29/40, 95 % CI: 58.7–86.3 %). The rate in the PPS was 72.7 % (24/33; 95 % CI: 57.5–87.9 %).

Table 2 Clinical efficacy rates

	ITT ($n = 40$)		PPS ($n = 33$)	
	Palpation	MRI/CT	Palpation	MRI/CT
CR	23 (57.5 %)	24 (60.0 %)	22 (66.7 %)	22 (66.7 %)
PR	6 (15.0 %)	12 (30.0 %)	4 (12.1 %)	9 (27.3 %)
SD	1 (2.5 %)	2 (5.0 %)	0 (0.0 %)	2 (6.1 %)
PD	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)
NE	10 (25.0 %)	2 (5.0 %)	7 (21.2 %)	0 (0.0 %)
Objective response rate[a]	29 (72.5 %; 95 % CI 56.1–85.4)	36 (90.0 %; 95 % CI 76.3–97.2)	26 (78.8 %; 95 % CI 61.1–91.0)	31 (93.9 %; 95 % CI 79.8–99.3)

ITT intent-to-treat, *PPS* per-protocol set, *MRI* magnetic resonance imaging, *CT* computed tomography, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *NE* not evaluable

[a] CR + PR

Table 3 pCR rates

	ITT ($n = 40$)	PPS ($n = 33$)	Weakly ER-positive ($n = 7$)
ypT0 ypN0	14 (35.0 %)	13 (39.4 %)	1 (14.3 %)
ypT0/Tis ypN0	19 (47.5 %)	18 (54.5 %)	3 (42.9 %)
near pCR (Grade 2) ypN0	3 (7.5 %)	2 (6.1 %)	0
QpCR ypN0	22 (55.0 %)	20 (60.6 %)	0

pCR pathologic complete response, *ITT* intent-to-treat, *PPS* per-protocol set, *QpCR* quasi-pCR

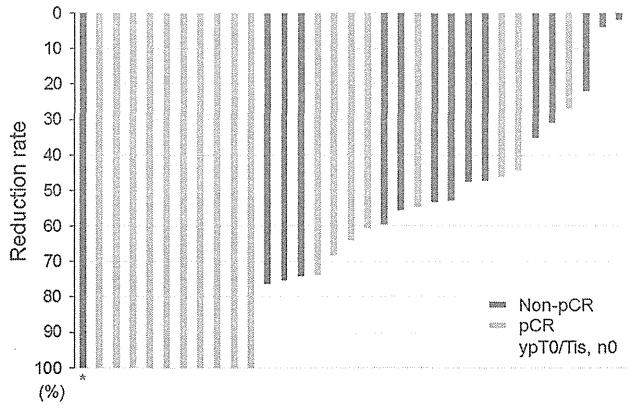

Fig. 2 Tumor response rates. Reduction in lesion size from baseline (%) to the time of mPCX therapy completion in individual patients. *This patient achieved ypT0/Tis in the breast, but an invasive tumor was found in the axillary lymph node. *pCR* pathologic complete response

Toxicity

Adverse events occurring during the study are presented in Table 4.

Grade ≥3 hematologic adverse events included leukopenia in 25 % (10/40), neutropenia in 35 % (14/40), and anemia in 5 % (2/40) of patients. Non-hematologic toxicities classified as Grade ≥3 included palmar-plantar erythrodysesthesia syndrome in 8 % (3/40) of patients, while nausea, vomiting, diarrhea, and peripheral sensory neuropathy occurred in one patient each. Interstitial pneumonia occurred in two patients (Grade 1) during mPCX therapy. No subjective symptoms were found, and the disease was only identifiable on imaging. The clinical signs of the disease resolved after observation and steroid therapy. Both patients successfully underwent postoperative FEC therapy.

One serious adverse event (pulmonary artery thrombosis) was detected after mPCX in one patient based on imaging findings. The patient had no symptoms and no reduction in oxygen saturation. A causal relationship with the study drug was ruled out based on the attending physician's judgment and the patient continued FEC chemotherapy, thereafter undergoing surgery.

Discussion

This study included women with primary TNBC or breast cancer with low ER/PgR expression, which are often associated with an unfavorable prognosis. New treatment options are necessary. To reduce the likelihood of disease recurrence and prolong the survival of patients with breast cancer, it is necessary to add other strategies to standard care. Patients with ER-positive and/or HER2-positive breast cancer may benefit from targeted therapies, such as endocrine therapy and anti-HER2 therapy. Unfortunately, there are few options for TBNC, and the currently available chemotherapies are somewhat limited. Therefore, it is essential to develop new treatment strategies for this disease. Although some novel agents are under development, we are focusing on metronomic chemotherapy based on a combination of approved anticancer drugs. The concept of metronomic chemotherapy

Table 4 Adverse events according to grade

$n = 40$	Grade 1 or 2	Grade 3 or 4
Hematologic toxicity		
Anemia (hemoglobin)	33 (83 %)	2 (5 %)
White blood cell count decreased	26 (65 %)	11 (28 %)
Neutrophil count decreased	21 (53 %)	14 (35 %)
Platelet count decreased	6 (15 %)	0
Non-hematologic toxicity		
Peripheral sensory neuropathy	30 (75 %)	1 (3 %)
Palmar-plantar erythrodysesthesia syndrome (HFS)	28 (70 %)	3 (8 %)
Nausea	28 (70 %)	1 (3 %)
Inflammation of the mucus membranes in the mouth	23 (58 %)	0
Alanine aminotransferase increased	22 (55 %)	1 (3 %)
Pyrexia	21 (53 %)	0
Nail changes[a]	20 (50 %)	0
Constipation	20 (50 %)	0
Aspartate aminotransferase increased	19 (48 %)	0
Vomiting	11 (28 %)	1 (3 %)
Diarrhea	9 (23 %)	1 (3 %)
Nail loss	7 (18 %)	0
Arthralgia	6 (15 %)	0
Myalgia	6 (15 %)	0
Eruption	4 (10 %)	0
Creatinine increased	2 (5 %)	0
Hemorrhoids	3 (8 %)	0
Blood bilirubin increased	2 (5 %)	0
Allergic reaction	2 (5 %)	0
Peripheral motor neuropathy	2 (5 %)	0
Dizziness (exertional)	2 (5 %)	0
General malaise	2 (5 %)	0
Interstitial pneumonia	2 (5 %)	0
Febrile neutropenia	0	12 (30 %)

[a] Adverse events were assessed based on the common terminology criteria for adverse events (CTCAE) version 4.0 except for "nail changes" (CTCAE version 3.0)

was based on the expectation that their anti-angiogenic effects would be associated with a reduced incidence of toxicities and avoiding drug resistance [17]. The evidence accumulated to date suggests that metronomic chemotherapy may have several new mechanisms of action, including restoration of the patient's anticancer immune response and the induction of tumor dormancy [42]. Although the results of phase III studies of metronomic chemotherapy have not yet been published, several recent studies have revealed that metronomic chemotherapy may be clinically beneficial and safe for a broad range of tumors [21–25], and this was further confirmed in a systematic literature analysis [42].

In this study, we applied the metronomic concept to PCX therapy, the first time this has been done with a combination of three drugs. The RDI for paclitaxel in the mPCX phase was almost 90 %, and the toxicities at this intensity were not serious; therefore, the combination showed good tolerability, similar to that for standard weekly paclitaxel. The metronomic PCX followed by standard FEC regimen resulted in pCR rates of 37.5 and 54.5 % in the ITT population and PPS, respectively. These values were higher than those of conventional anthracycline (A) chemotherapy (around 20 %) [5–8], taxanes (T) alone (from 5 to 12 %) [5, 28], and standard chemotherapy with a sequential combination of A and T for TNBC (around 30 %) [5]. About 5–10 % of patients with triple-negative breast cancer experience tumor progression during neoadjuvant chemotherapy because of drug resistance. Tumor progression may be found by chance because none of the subjects whose tumor progressed during neoadjuvant chemotherapy (Table 2) in this study received metronomic PCX followed by FEC.

We also analyzed the results of mPCX in breast cancer patients with weakly positive ER. Invasive breast cancer disappeared in four of these patients (ypT0/Tis) after mPCX-EFC therapy, of whom three patients were axillary lymph node negative (ypN0).

Among 35 patients who completed four cycles of mPCX, 11 achieved CR with a complete loss of lesions. Of these 11 patients, 10 had a pCR.

The positive outcomes outlined here may result from the favorable efficacy profile of metronomic mPCX itself, combined with the reduced toxicity of this dosing regimen. It is possible that a CR after the first mPCX could be a surrogate marker of pCR. Furthermore, pCR could be expected after clinical CR (cCR) in response to mPCX, while surgery in patients with cCR after mPCX could lead to pCR with good prognosis.

TNBC includes a range of phenotypes. Unfortunately, we do not yet know which subtypes, for example high or low proliferative subtypes, are the most suitable candidates for metronomic chemotherapy. However, considering the anti-angiogenic mechanism of metronomic chemotherapy, its efficacy might be independent of the tumor's proliferative capacity. To improve the pCR rate for TNBC, carboplatin and/or bevacizumab were used in combination with taxanes in two recent trials. The GeparSixto-GBG 66 and CALGB/Alliance 40603 clinical trials [43, 44] revealed that the use of carboplatin and/or bevacizumab increased the pCR rate to 50–60 %, similar to the rate for mPCX followed by FEC in our study. Regarding adverse events, carboplatin was associated mild or serious bone marrow suppression. Some patients given carboplatin required treatment with granulocyte colony-stimulating factor and some patients experienced grade 3/4 anemia and/or thrombocytopenia. By contrast, mPCX was not associated with

additional serious adverse events, which suggests it is associated with fewer toxicities and improved efficacy compared with other regimens. We are now planning to conduct translational studies focusing on a variety of biomarkers. These studies should reveal which tumor subtypes are suitable candidates for metronomic chemotherapy. We are also planning another clinical trial to confirm the usefulness of metronomic chemotherapy for TNBC.

Based on the results of Fig. 2, the tumor response during mPCX might be predictive of pCR. Almost all of the patients with cCR after mPCX achieved CpCR after FEC. This may help us to predict which patients may not require an anthracycline, thus avoiding the associated risk of cardiac toxicity. This may also help us identify which patients may not require surgery to remove the original tumor. Importantly, if a CR is achieved after mPCX therapy, the anthracycline regimen may be discontinued in patients with a pCR, which could be particularly beneficial because of the risk of cardiotoxicity associated with anthracyclines. With mPCX, we may therefore have access to a new treatment option in which potentially cardiotoxic FEC can be avoided, at least in some patients. However, if pCR is not achieved with metronomic mPCX therapy alone (without subsequent anthracycline-based chemotherapy) postoperative anthracycline-based chemotherapies may still be administered. The efficacy of postoperative chemotherapy with anthracyclines was demonstrated by Bear et al. [42], who found no differences in prognosis between patients treated preoperatively with anthracycline plus docetaxel and those treated preoperatively with anthracycline and postoperatively with docetaxel. Patients enrolled in the present study are now being followed up to determine whether pCR after four cycles of metronomic mPCX allows the avoidance of subsequent FEC chemotherapy. Notably, breast conservation surgery was possible in six patients (40 %) who were scheduled to undergo total mastectomy, while 23 (92.0 %) of patients underwent BCS as planned.

The incidence of Grade $\geq$3 non-hematologic adverse events was generally low and similar to that reported for metronomic cyclophosphamide and capecitabine [18, 44] or cyclophosphamide/methotrexate [24]. Grade $\geq$3 hematologic events occurred in 10–25 % of patients, which is somewhat higher than that reported for metronomic cyclophosphamide/methotrexate [24]. However, only one serious adverse event occurred, which was not considered related to the study drug. The rate of compliance was also high, based on the high RDI rates.

Some limitations of this study warrant mention. First, the sample size was small (only 40 patients), although it was adequately powered based on the planned sample size. Second, the pCR rate may be further improved by the combination of a PARP inhibitor or bevacizumab with metronomic mPCX [45, 46], although the benefits of adding bevacizumab would need to be balanced against the possibility of a higher incidence of grade 3 or 4 toxicities [47].

In conclusion, metronomic PCX followed by FEC chemotherapy was associated with a high pCR rate and low toxicity in patients with TNBC. Further studies of this regimen in larger numbers of patients are warranted.

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Conflict of interest Norikazu Masuda has received honoraria from Chugai. Satoshi Morita has received honoraria and research funding from Chugai. Masakazu Toi has received honoraria from Chugai and research funding from Chugai and BMS. All other authors have no conflicts of interest to declare.

Ethical standard The experiments performed in this study comply with current Japanese law.

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CLINICAL TRIAL

Survival of HER2-positive primary breast cancer patients treated by neoadjuvant chemotherapy plus trastuzumab: a multicenter retrospective observational study (JBCRG-C03 study)

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Abstract We investigated the disease-free survival (DFS) of HER2-positive primary breast cancer patients treated with neoadjuvant chemotherapy plus trastuzumab, as well as predictive factors for DFS and pathologic response. Data from 829 female patients treated between 2001 and 2010 were collected from 38 institutions in Japan. Predictive factors were evaluated using multivariate analyses. The 3-year DFS rate was 87 % [95 % confidence interval (CI) 85–90]. The pathologic complete response (pCR: ypT0/is + ypN0) rate was 51 %. The pCR rate was higher in the ER/PgR-negative patients than in the ER/PgR-positive patients (64 vs. 36 %, $P < 0.001$). Patients with pCR showed a higher DFS rate than patients without pCR (93 vs. 82 %, $P < 0.001$). Multivariate analysis revealed three independent predictors for poorer DFS: advanced nodal stage [hazard ratio (HR) 2.63, 95 % CI 1.36–5.21, $P = 0.004$ for cN2–3 vs. cN0], histological/nuclear grade 3 (HR 1.81, 95 % CI 1.15–2.91, $P = 0.011$), and non-pCR (HR 1.98, 95 % CI 1.22–3.24, $P = 0.005$). In the ER/PgR-negative dataset, non-pCR (HR 2.63, 95 % CI 1.43–4.90, $P = 0.002$) and clinical tumor stage (HR 2.20, 95 % CI 1.16–4.20, $P = 0.017$ for cT3–4 vs. cT1–2) were independent predictors for DFS, and in the ER/PgR-positive dataset, histological grade of 3 (HR 3.09, 95 % CI 1.48–6.62, $P = 0.003$), clinical nodal stage (HR 4.26, 95 % CI 1.53–13.14, $P = 0.005$ for cN2–3 vs. cN0), and young age (HR 2.40, 95 % CI 1.12–4.94, $P = 0.026$ for ≤40 vs. >40) were negative predictors for DFS. Strict pCR (ypT0 + ypN0) was an independent predictor for DFS in

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both the ER/PgR-negative and -positive datasets (HR 2.66, 95 % CI 1.31–5.97, $P = 0.006$ and HR 3.86, 95 % CI 1.13–24.21, $P = 0.029$, respectively). These results may help assure a more accurate prognosis and personalized treatment for HER2-positive breast cancer patients.

Keywords Breast cancer · HER2 · Neoadjuvant chemotherapy · Pathologic complete response · Prognostic factors · Trastuzumab

Introduction

Amplification or overexpression of human epidermal growth factor receptor-2 (HER2) is associated with a high risk of breast cancer recurrence and metastasis [1]. Adjuvant use of cytotoxic chemotherapy and trastuzumab, a recombinant humanized monoclonal antibody that targets HER2, improves the overall survival (OS) and disease-free survival (DFS) of patients with HER2-positive primary breast cancer [2, 3].

Neoadjuvant chemotherapy (NAC) reduces tumor size, which improves the rate of breast-conserving surgery, and provides information about chemosensitivity that helps with the design of postoperative therapy. Several meta-analyses have revealed that patients with a pathologic complete response (pCR) after NAC had higher survival rates than those without pCR, indicating that pCR represents a surrogate prognostic indicator [4–6].

Adding trastuzumab to NAC doubles the rate of pCR in patients with HER2-positive primary breast cancer [7–9]. The NOAH trial showed better 3-year event-free survival for chemotherapy plus trastuzumab versus chemotherapy alone [8]. In the TECHNO trial, patients with pCR after NAC plus trastuzumab showed better 3-year DFS than patients without pCR [10]; however, predictors for pCR and survival after treatment are unknown.

This multicenter retrospective study investigated the survival after NAC with trastuzumab among patients with HER2-positive primary breast cancer in efforts to identify predictive factors.

Patients and methods

Patients

In this multicenter retrospective cohort study, the inclusion criteria were female sex, histologically confirmed HER2-positive invasive breast cancer diagnosed between 2001 and 2010, no distant metastasis, age 20–70 years, and received NAC containing trastuzumab. Eligible patients were identified from the institutional databases. Data were managed by the data center of the Japan Breast Cancer Research Group (JBCRG).

The study protocol was approved by the Institutional Review Board at Kyoto University Hospital and participating institutions. All patient data were anonymized and

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allocated numbers according to Japanese ethics guidelines for epidemiologic research.

Pathological assessment

Pathology specialists at each institution performed the pathological investigation. HER2-positive status was defined as 3+ overexpression by immunohistochemical testing or HER2 amplification by fluorescent in situ hybridization (HER2/CEP17 ratio $\geq$2.0). At each institution, surgical specimens obtained following NAC were serially sectioned, stained with hematoxylin and eosin (H&E), and diagnosed by experienced pathologists. pCR was defined as the absence of residual invasive cancer cells in the breast and axillary lymph nodes (ypT0/is + ypN0). Strict pCR (spCR), another pCR definition, was defined as no invasive and non-invasive residuals in the breast and axillary nodes (ypT0 + ypN0).

Statistical analysis

All survival outcomes were measured from the date of starting NAC to the date of first event. The primary survival outcome was DFS defined as time to occurrence of recurrence, secondary malignancy (including contralateral breast cancer, hematological malignancy, and sarcoma), or death as a result of any cause. Secondary survival outcomes were OS defined as time to death as a result of any cause, distant recurrence-free survival (DRFS) defined as time to any recurrence except for ipsilateral breast or regional lymph node, and death as a result of any cause.

The Kaplan–Meier method was used to estimate survival outcomes. χ^2 tests for categorical data and log-rank tests for time-to-event endpoints provided two-sided P values, and P values < 0.05 were considered statistically significant. Cox proportional hazards regression analysis was used to estimate hazard ratios (HRs) and 95 % confidence intervals (CIs). Logistic regression was used to estimate odds ratios (ORs) and 95 % CIs. Covariates used in the multivariate model were age, body mass index, clinical tumor stage, clinical nodal stage, estrogen receptor (ER)/progesterone receptor (PgR) status, histological/nuclear grade, pCR/spCR, surgery type, radiation therapy, adjuvant hormonal therapy, adjuvant chemotherapy, and adjuvant trastuzumab. Menopausal status was not included in the model because of collinearity with age. Patients with missing data were excluded from the multivariate analysis (e.g., patients whose adequate pathologic responses were not confirmed due to insufficient local therapy or lack of information regarding local therapy type). All statistical analyses were performed using JMP® (ver. 10.0.2, SAS Institute Inc. Cary, NC, USA). All analyses were supervised by a statistician (SM).

Results

Patient characteristics

Data of 829 patients from 38 institutions in Japan were collected. Among them, 53 did not meet the inclusion criteria and were excluded, leaving a total of 776 patients for analysis (whole dataset). HER2-positive tumors could be subdivided into ER/PgR positive and negative, and we therefore divided the patients into an ER/PgR-positive dataset ($N = 334$) and ER/PgR-negative dataset ($N = 439$) and also performed the analyses for each dataset (Fig. 1).

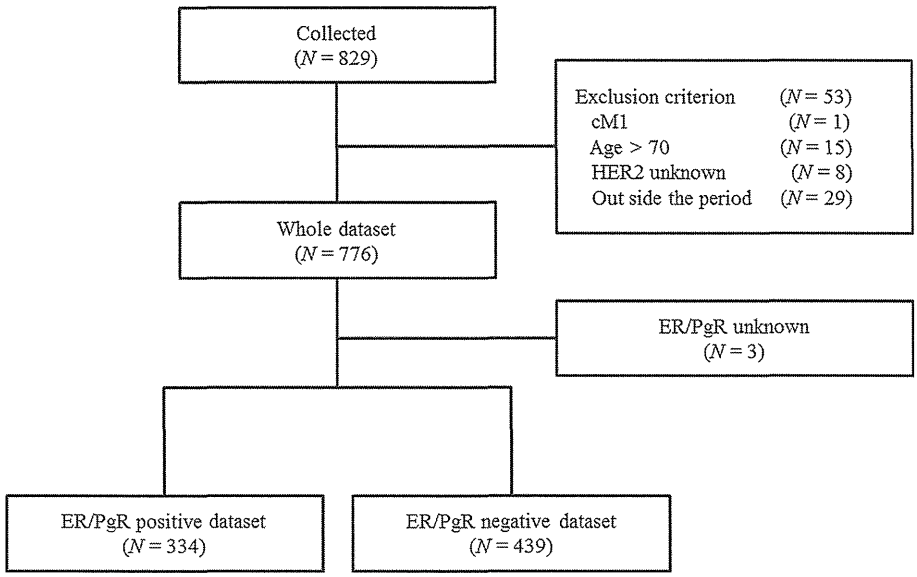

Fig. 1 Flowchart of data collection and analysis

Table 1 Patient, disease, and treatment characteristics

Factors	*n*	(%)
All cases	776	(100)
Age		
Median (min–max)	53	(25–70)
BMI		
Median (min–max)	22.0	(15.0–47.3)
Unknown	2	(0.3)
Menopausal status		
Pre-menopausal	335	(43.2)
Post-menopausal	422	(54.4)
Unknown	19	(2.4)
Clinical tumor size		
T1b	9	(1.2)
T1c	77	(9.9)
T2	476	(61.3)
T3	122	(15.7)
T4	91	(11.7)
Unknown	1	(0.1)
Clinical nodal status		
N0	252	(32.5)
N1	366	(47.2)
N2	103	(13.3)
N3	54	(7)
Unknown	1	(0.1)
ER/PgR status		
Positive	334	(43)
Negative	439	(56.6)
Unknown	3	(0.4)
Histological/nuclear grade		
1	107	(13.8)
2	184	(23.7)
3	350	(45.1)
Unknown	135	(17.4)
NAC regimen		
Anthracycline and taxane	676	(87.1)
Taxane only	78	(10.1)
Anthracycline only	7	(0.9)
Others	1	(0.1)
Unknown	14	(1.8)
Local therapy		
Mastectomy + XRT	96	(12.4)
Mastectomy alone	181	(23.3)
BCS + XRT	449	(57.9)
BCS alone	44	(5.7)
Needle biopsy + XRT	1	(0.1)
Needle biopsy alone	1	(0.1)
Unknown	4	(0.5)
pCR (ypT0/is + ypN0)		
Yes	399	(51.4)
No	365	(47)
Unknown	12	(1.5)
spCR (ypT0 + ypN0)		
Yes	240	(30.9)
No	525	(67.7)
Unknown	11	(1.4)
Adjuvant hormonal therapy		
Yes	281	(36.2)
No	440	(56.7)
Unknown	55	(7.1)
Adjuvant trastuzumab therapy		
Yes	697	(89.8)
No	65	(8.4)
Unknown	14	(1.8)
Adjuvant chemotherapy		
Yes	45	(5.8)
No	720	(92.8)
Unknown	11	(1.4)

BMI body mass index, *ER/PgR* estrogen receptor/progesterone receptor, *NAC* neoadjuvant chemotherapy, *XRT* radiation therapy, *BCS* breast-conserving surgery, *pCR* pathologic complete response

Baseline characteristics and treatment of the whole dataset are summarized in Table 1. Median age was 53 (range 25–70) years. Most patients had tumor stage T2 (61 %) and were clinically node positive (67 %). ER and PgR were negative in 57 % of the patients. Most patients received anthracycline- and taxane-containing chemotherapy (87 %), and trastuzumab was administered concurrently with taxane (80 %). Breast-conserving surgery was performed in 64 % of the patients, most of whom (91 %) received radiation therapy. Radiation therapy was performed in 35 % of the patients who received mastectomy. Adjuvant hormonal therapy was performed in 86 % of the ER/PgR-positive patients. Most patients received adjuvant trastuzumab (90 %).

Clinical outcomes

The median follow-up period was 42 (interquartile range 30–58) months. For the whole dataset, the 3-year DFS rate was 87 % (95 % CI 85–90) (Fig. 2a). 3-year OS and DRFS were 97 % (95 % CI 96–98) and 91 % (95 % CI 89–93), respectively. pCR was achieved in 399 (51 %) patients and spCR in 240 (31 %) patients.

The 3-year DFS rate was almost the same among patients in the ER/PgR-positive and -negative datasets (87 vs. 88 %, $P = 0.888$) (Fig. 2B). The pCR and spCR rates were higher in the ER/PgR-negative patients than in the ER/PgR-positive

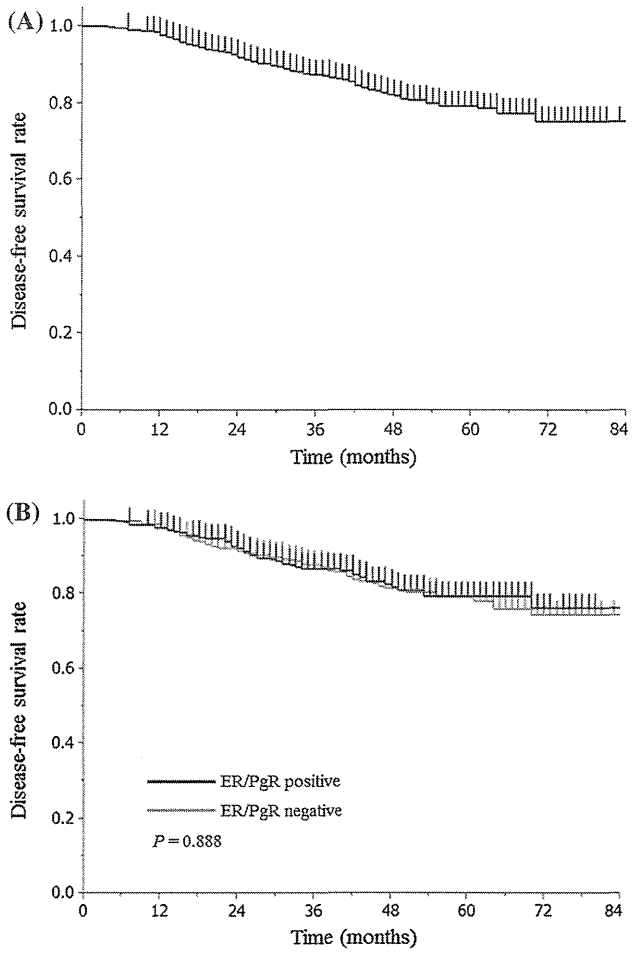

Fig. 2 DFS curves of the **a** whole dataset and **b** ER/PgR-positive and -negative datasets

patients (64 vs. 36 % for pCR, $P < 0.001$; 38 vs. 23 % for spCR, $P < 0.001$, respectively).

Prognostic factors for survival outcomes

The results of Cox proportional hazard regression performed to evaluate the prognostic effect of baseline characteristics and pathologic tumor response to NAC with trastuzumab are shown in Table 2. In the whole dataset, independent predictors for poorer DFS were advanced clinical nodal stage (adjusted HR 2.63, 95 % CI 1.36–5.21, $P = 0.004$ for cN2–3 vs. cN0; adjusted HR 1.64, 95 % CI 0.91–3.09, $P = 0.100$ for cN1 vs. cN0), histological/nuclear grade 3 (adjusted HR 1.81, 95 % CI 1.15–2.91, $P = 0.011$), and failure to achieve pCR (adjusted HR 1.98, 95 % CI 1.22–3.24, $P = 0.005$). Neither age nor ER/PgR status was an independent predictor for DFS. Multivariate analysis including spCR yielded the same results. The DFS rate was higher among patients with pCR than those without pCR (93 vs. 82 %, $P < 0.001$) (Fig. 3a). Patients who achieved spCR had a higher DFS rate than those who did not (96 vs. 84 %, $P < 0.001$) (Fig. 3b).

In the ER/PgR-positive dataset, independent predictors for poorer DFS were advanced clinical nodal stage, histological/nuclear grade 3, young age (≤40), and not achieving spCR. pCR was not an independent predictor for DFS on multivariate analysis (Table 2; Fig. 3c, d). For the ER/PgR-negative dataset, clinical tumor stage and both pCR and spCR were independent predictors for DFS (Table 2; Fig. 3e, f).

Predictors for other survival outcomes are listed in Supplementary Table S1. Predictors for OS were clinical nodal stage, histological/nuclear grade, and spCR, but pCR was not an independent predictor. Predictors for DRFS were clinical nodal stage, histological/nuclear grade, young age, pCR, and spCR.

Predictive factors for pCR

The association of baseline characteristics with pCR/spCR following NAC plus trastuzumab was evaluated by multivariate logistic regression (Table 3). In the whole dataset, independent predictors for pCR were negative ER/PgR status (adjusted OR 3.42, 95 % CI 2.42–4.86, $P < 0.001$) and clinical tumor stage T1–2 compared with T3–4 (adjusted OR 1.88, 95 % CI 1.27–2.79, $P = 0.002$). Histological/nuclear grade 3 showed a statistically marginal association with pCR (adjusted OR 1.39, 95 % CI 0.99–1.95, $P = 0.060$). The same factors were selected as independent predictors in the multivariate model for spCR.

In the ER/PgR-positive dataset, clinical tumor stage was a predictor for pCR and spCR. In the ER/PgR-negative dataset, clinical tumor stage was an independent predictor for both pCR and spCR. Histological/nuclear grade was marginally predictive of pCR and spCR.

Discussion

In this analysis, we assessed survival after NAC plus trastuzumab among patients with HER2-positive breast cancer. Although clinical nodal status, histological/nuclear grade, and pCR/spCR were independent predictors for DFS, the prognostic impact differed depending on ER/PgR status. pCR was a predictor for DFS particularly in patients with ER/PgR-negative tumor, and spCR—a stricter definition of pCR—was an independent prognostic factor regardless of ER/PgR status.

Our data included more patients with clinical tumor stage T2 or higher (89 %) and clinically node positive (67 %). In this population, a 3-year DFS rate of 87 % was relatively good; however, a considerable number of patients experienced disease relapse during the follow-up period. Risk factors associated with disease relapse need to

Table 2 Adjusted hazard ratios of factors predicting DFS

Factor	pCR (ypT0/is + ypN0)			spCR (ypT0 + ypN0)		
	HR	95 % CI	*P* value	HR	95 % CI	*P* value
Whole dataset						
Age						
≤40 vs. >40	1.67	(0.95–2.81)	0.074	1.63	(0.93–2.75)	0.088
BMI						
25≤ vs. <22	1.31	(0.74–2.24)	0.351	1.31	(0.74–2.24)	0.348
22≤ , <25 vs. <22	0.96	(0.56–1.61)	0.891	1.00	(0.58–1.67)	0.993
Clinical tumor size						
T3–4 vs. T1–2	1.53	(0.93–2.49)	0.093	1.42	(0.87–2.32)	0.160
Clinical nodal status						
N2–3 vs. N0	2.63	(1.36–5.21)	0.004	2.58	(1.34–5.12)	0.004
N1 vs. N0	1.64	(0.91–3.09)	0.100	1.73	(0.96–3.26)	0.070
ER/PgR						
Negative vs. positive	0.97	(0.47–2.08)	0.933	0.93	(0.46–1.96)	0.842
Histological/Nuclear grade						
3 vs. 1&2	1.81	(1.15–2.91)	0.011	1.77	(1.12–2.84)	0.014
pCR/spCR						
Non-pCR vs. pCR	1.98	(1.22–3.24)	0.005	2.90	(1.57–5.90)	<0.001
ER/PgR-positive dataset						
Age						
≤40 vs. >40	2.40	(1.12–4.94)	0.026	2.33	(1.08–4.80)	0.031
BMI						
25≤ vs. <22	1.49	(0.63–3.38)	0.354	1.54	(0.66–3.45)	0.313
22≤, <25 vs. <22	0.69	(0.25–1.67)	0.419	0.69	(0.25–1.68)	0.433
Clinical tumor size						
T3–4 vs. T1–2	0.83	(0.35–1.88)	0.653	0.69	(0.28–1.62)	0.399
Clinical nodal status						
N2–3 vs. N0	4.26	(1.53–13.14)	0.005	4.54	(1.62–14.13)	0.004
N1 vs. N0	2.55	(0.99–7.43)	0.053	2.83	(1.08–8.39)	0.034
Histological/Nuclear grade						
3 vs. 1&2	3.09	(1.48–6.62)	0.003	3.14	(1.49–6.85)	0.003
pCR/spCR						
Non-pCR vs. pCR	1.20	(0.57–2.69)	0.634	3.86	(1.13–24.21)	0.029
ER/PgR-negative dataset						
Age						
≤40 vs. >40	0.95	(0.35–2.18)	0.913	1.01	(0.38–2.28)	0.979
BMI						
25≤ vs. <22	0.94	(0.39–2.05)	0.886	0.97	(0.40–2.11)	0.942
22≤, <25 vs. <22	1.10	(0.56–2.08)	0.774	1.10	(0.56–2.08)	0.779
Clinical tumor size						
T3–4 vs. T1–2	2.20	(1.16–4.20)	0.017	2.11	(1.11–4.04)	0.024
Clinical nodal status						
N2–3 vs. N0	2.04	(0.85–5.07)	0.112	1.73	(0.73–4.27)	0.217
N1 vs. N0	1.49	(0.70–3.38)	0.306	1.39	(0.66–3.13)	0.398
Histological/Nuclear grade						
3 vs. 1&2	1.33	(0.74–2.48)	0.354	1.29	(0.72–2.41)	0.393
pCR/spCR						
Non-pCR vs. pCR	2.63	(1.43–4.90)	0.002	2.66	(1.31–5.97)	0.006

BMI body mass index, *ER/PgR* estrogen receptor/progesterone receptor, *pCR* pathologic complete response, *spCR* strict pathologic complete response, *HR* hazard ratio

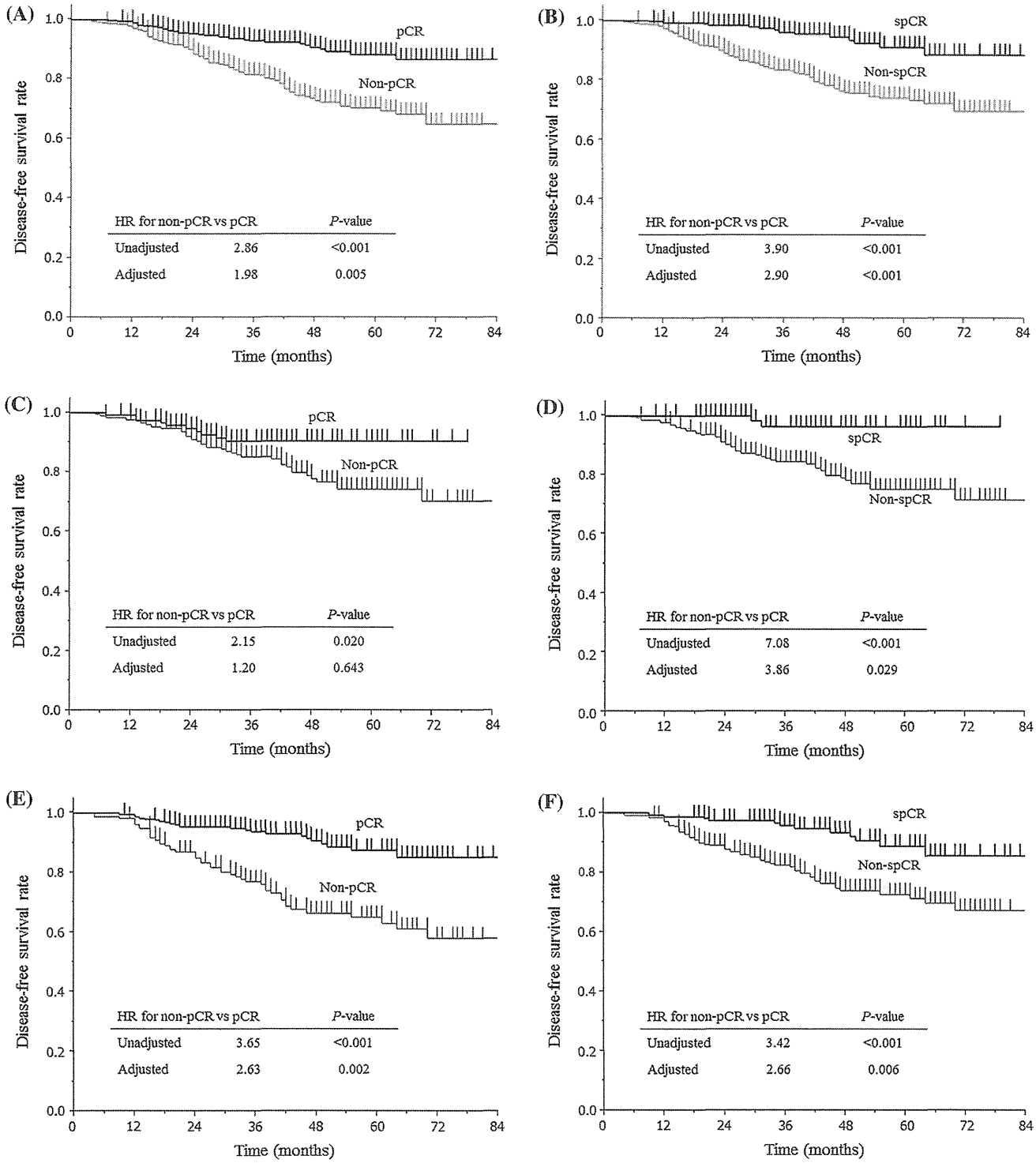

Fig. 3 DFS curves of patients with pCR (ypT0/is + ypN0) versus non-pCR in the **a** whole dataset, **c** ER/PgR-positive dataset, and **e** ER/PgR-negative dataset. DFS curves of patients with spCR (ypT0 + ypN0) versus non-spCR in the **b** whole dataset, **d** ER/PgR-positive dataset, and **f** ER/PgR-negative dataset

be clarified to conduct a clinical trial aimed at improving these patients' prognosis.

In two phase-III trials in which patients with HER2-positive disease were randomly allocated to NAC with trastuzumab or NAC only, the addition of trastuzumab to NAC resulted in a higher pCR rate and improved DFS [8, 11]. The pCR rate in our study (51 %) is comparable to those reported in previous trials of NAC with trastuzumab

Table 3 Adjusted odds ratios of factors predicting pCR

Factor	pCR (ypT0/is + ypN0)			spCR (ypT0 + ypN0)		
	OR	95 % CI	*P* value	OR	95 % CI	*P* value
Whole dataset						
Age						
>40 vs. ≤40	0.97	(0.60–1.58)	0.907	1.45	(0.84–2.63)	0.191
BMI						
25≤ vs. <22	1.22	(0.78–1.91)	0.388	1.31	(0.80–2.11)	0.280
22≤, <25 vs. <22	1.38	(0.94–2.04)	0.100	1.47	(0.98–2.21)	0.062
Clinical tumor size						
T1–2 vs. T3–4	1.88	(1.27–2.79)	0.002	2.16	(1.39–3.41)	0.001
Clinical nodal status						
N0 vs. N2–3	0.65	(0.40–1.07)	0.093	0.98	(0.57–1.71)	0.942
N1 vs. N2–3	0.83	(0.53–1.31)	0.435	1.44	(0.88–2.39)	0.152
ER/PgR status						
Negative vs. positive	3.42	(2.42–4.86)	<0.001	2.27	(1.55–3.35)	<0.001
Histological/Nuclear grade						
3 vs. 1&2	1.39	(0.99–1.95)	0.060	1.29	(0.90–1.88)	0.169
ER/PgR-positive dataset						
Age						
>40 vs. ≤40	0.74	(0.40–1.39)	0.343	1.22	(0.56–2.89)	0.622
BMI						
25≤ vs. <22	1.65	(0.85–3.20)	0.140	1.27	(0.56–2.81)	0.559
22≤, <25 vs. <22	1.43	(0.77–2.61)	0.253	1.46	(0.71–2.97)	0.296
Clinical tumor size						
T1–2 vs. T3–4	1.76	(0.94–3.43)	0.078	2.95	(1.28–7.72)	0.010
Clinical nodal status						
N0 vs. N2–3	0.98	(0.46–2.11)	0.954	0.89	(0.36–2.32)	0.810
N1 vs. N2–3	0.80	(0.39–1.67)	0.547	0.93	(0.39–2.35)	0.869
Histological/Nuclear grade						
3 vs. 1&2	1.22	(0.73–2.05)	0.454	1.00	(0.54–1.86)	0.991
ER/PgR-negative dataset						
Age						
>40 vs. ≤40	1.43	(0.68–2.94)	0.344	1.73	(0.80–4.08)	0.170
BMI						
25≤ vs. <22	0.95	(0.52–1.76)	0.871	1.29	(0.69–2.36)	0.422
22≤, <25 vs. <22	1.35	(0.81–2.27)	0.248	1.47	(0.89–2.43)	0.132
Clinical tumor size						
T1–2 vs. T3–4	1.93	(1.17–3.20)	0.010	1.89	(1.13–3.24)	0.016
Clinical nodal status						
N0 vs. N2–3	0.48	(0.24–0.92)	0.027	0.98	(0.49–1.95)	0.943
N1 vs. N2–3	0.89	(0.48–1.61)	0.692	1.75	(0.97–3.26)	0.065
Histological/Nuclear grade						
3 vs. 1&2	1.53	(0.97–2.42)	0.068	1.50	(0.94–2.40)	0.087

BMI body mass index, *ER/PgR* estrogen receptor/progesterone receptor, *pCR* pathologic complete response, *spCR* strict pathologic complete response, *OR* odds ratio

(30–67 %) [7–10, 12–15]. In our study, ER/PgR status was the strongest predictor for pCR or spCR. Our results were consistent with those of two meta-analyses in which the pCR rate of NAC with trastuzumab was about 50 % for patients with ER/PgR-negative disease and 30 % for those with ER/PgR-positive disease [6, 16].

In the TECHNO trial, a phase-II trial of 217 patients with HER2-positive disease who received NAC with trastuzumab, failure to achieve pCR was a significant predictor for DFS in the multivariate analysis [10]. Kim et al. [12] retrospectively investigated the prognostic value of pCR using data from 229 patients with HER2-positive

tumor who were treated with NAC with trastuzumab. They reported that pCR, clinical tumor stage, and lymphovascular invasion were independent predictors for DFS. In our study, pCR and spCR were predictors for DFS; in addition, conventional prognostic factors such as nodal stage and histological/nuclear grade were predictors for DFS.

In this study, the association of age with DFS was not statistically significant in the whole dataset, consistent with the results of the TECHNO trial and Kim et al. Partridge et al. [17] reported that young age was not associated with worse DFS in patients with HER2-positive disease using large cohort data from the HERA trial. When we divided the patients into ER/PgR-positive and -negative groups, multivariate analysis showed that young age (age $\leq$40) was an independent predictor for poorer DFS in the ER/PgR-positive dataset. Our result was consistent with earlier studies showing that younger age is an independent predictor for worse DFS, especially in patients with ER/PgR-positive disease [18, 19].

After dividing the patients into ER/PgR-positive and -negative datasets, we performed multivariate analysis for DFS using each dataset. About 30–40 % of HER2-enriched subtype tumors are reported to be ER positive [20, 21]. Among clinically HER2-positive tumors, up to 60 % are classified as the HER2-enriched subtype, with the rest classified as luminal B, luminal A, or basal-like [22]. Adjuvant systemic therapy differs according to ER/PgR status [23]. Therefore, it seemed reasonable to perform the analysis based on ER/PgR status; however, the results should be interpreted carefully because of the relatively small event rate in each dataset.

In relation to the two aforementioned meta-analyses, pooled analysis from the German study group [6] indicated that pCR was a prognostic factor for the HER2-positive non-luminal subgroup, but not for those in the HER2-positive luminal subgroup. In the meta-analysis from the Collaborative Trials in Neoadjuvant Breast Cancer (CTNeoBC) [16], there was a stronger association of pCR with event-free survival in the HER2-positive non-luminal subgroup compared with those in the HER2-positive luminal subgroup. In our study, pCR was an independent predictor for DFS in the ER/PgR-negative dataset, but not ER/PgR-positive dataset, and spCR was an independent predictor for DFS regardless of ER/PgR status.

The limitations of this study include its retrospective design. Adjustment using multivariate analysis is mandatory to minimize selection bias. The relatively short observation period may also limit the interpretation of our results. The median follow-up period of our study (42 months) covered the time when recurrence risk is high in HER2-positive disease [24]. Strength of our study was the large number of patients, which allowed us to conduct multivariate analysis separately according to ER/PgR status.

In conclusion, pCR/spCR, nodal status, and grade were predictors for DFS in patients with HER2-positive disease treated with NAC plus trastuzumab. Response to therapy and prognostic impact of the factors differed according to ER/PgR status. Our results may help identify patients who are not likely to achieve pCR or whose outcome would otherwise be unfavorable. New treatment approaches, such as the incorporation of novel anti-HER2 drugs, are needed for patients with high-risk disease.

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Conflict of interest H. Iwata, S. Ohno, N. Masuda, and S. Morita received honorarium from Chugai Pharmaceutical Co., Ltd. N. Masuda received honorarium from Eisai Co., Ltd. M. Toi is currently conducting research sponsored by Chugai Pharmaceutical Co., Ltd. and received donation from Chugai Pharmaceutical Co., Ltd. All remaining authors have declared no conflicts of interest.

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