Additional effect of anthracycline in preoperative chemotherapy with a sequential anthracycline-containing regimen preceded by pertuzumab, trastuzumab and docetaxel combination therapy

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Abstract. The proper use of anthracycline-containing regimens in combination with anti-HER2-targeted therapy in a neoadjuvant setting for patients with HER2-positive breast cancer has not been resolved. Regimens preceded by anthracyclines have become the standard of care, and although the order has no significant impact on HER2-negative breast cancer, it is inconclusive as to whether a taxane-first sequence would have a similar effect on HER2-positive breast cancer. The present study aimed to investigate the benefit of a taxane-first sequence and of adriamycin and cyclophosphamide (AC) in patients with non-clinical complete response (non-cCR) to pertuzumab, trastuzumab and docetaxel (PTD). The present single-center prospective observational study was performed to investigate PTD followed by AC, and aimed to clarify the cCR rate after PTD alone and the pathological clinical response (pCR) rate after subsequent AC in patients without cCR after PTD alone. A total 24 patients were analyzed; of these, 14 achieved pCR (pCR rate, 58.3%). While four of 14 patients (28.6%) in the intention-to-treat population achieved pCR, nine of 14 patients (64.3%) achieved pCR with AC but not cCR after PTD. The median tumor reduction rate after four cycles of PTD was 58.9% (range, 20.8-100%) in all 24 patients, whereas the reduction rate after PTD-AC was 76.9% (range, 31.1-100%). Cardiac serious adverse events occurred in three patients (12.5%). In conclusion, a high pCR rate was observed for the taxane-first sequence. Patients were highly responsive to PTD, but some cases achieved additional antitumor effects after AC, which resulted in pCR without cCR after PTD alone. Since cardiotoxicity remains a significant problem, a higher risk-benefit treatment strategy is required to aim for AC omission. Trial registration number: UMIN000046338, name of registry: UMIN-CTR, date of registration: December 10, 2021.

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Introduction

HER2-positive breast cancer was originally a subtype with a poor prognosis due to its aggressiveness, but improvements in anti-HER2 therapy have dramatically reduced the frequency of recurrence and have prolonged overall survival (1). Perioperative chemotherapy, which includes trastuzumab (Tmab)- and pertuzumab (Pmab)-containing regimens for HER2-positive breast cancer, has become the standard of care when administered sequentially with anthracycline. In the NeoSphere study, the pathological complete response (pCR) rate was 45.8% after combination of Tmab and Pmab with docetaxel as neoadjuvant chemotherapy (NAC), which is a significantly higher pCR rate than that of the other three regimens investigated in this study; Tmab + Pmab, docetaxel + Tmab, and docetaxel + Pmab (2). As for the order of taxane and anthracycline, Wildiers *et al* reported that taxane-first regimens were favorable in terms of the relative dose intensity in an adjuvant setting (3). When preceded by taxanes, Neo-tAnGo showed a higher pCR rate between paclitaxel-epirubicin plus cyclophosphamide (EC) and EC-paclitaxel (20% vs. 15%, P=0.03), and no difference was observed in disease-free survival (DFS) [HR=0.82, 95% CI (0.63-1.06), P=0.12] (4). Iwata *et al* reported a high pCR rate with taxane-first regimens

(docetaxel-FEC) and confirmed these findings in a Japanese breast cancer series in which the safety profile was also found to be acceptable (5). These data suggested an advantage of a taxane-first strategy. As for the combination with anti-HER2 treatment, the FinHER trial showed that the combination of taxane preceded by anthracycline and T-mab prolonged distant DFS [docetaxel-FEC plus Tmab vs. no-Tmab, HR=0.32, 95% CI (0.12-0.98), P=0.029] (6). Preoperative chemotherapy with sequential Pmab plus Tmab plus docetaxel (PTD) followed by doxorubicin plus cyclophosphamide (AC) is now covered by insurance in Japan because of the negative disadvantages associated with the order of administration. Therefore, the taxane-first sequence with anti-HER2 targeted therapy in which PTD-AC might be considered one of the standard regimens for HER2-positive breast cancer is an option. However, the optimal clinical positioning of Pmab-containing regimens and anthracyclines is still debatable, and specifically, the omission of anthracyclines is still an important issue because of cardiotoxicity. There is another advantage of investigating the additional benefit of AC in patients with non-cCR after PTD, if a PTD-first sequence is planned. Herein to investigate the risk and benefit of additional AC and taxane-first regimens in HER2-positive breast cancer, we conducted a prospective observational study of neoadjuvant PTD-AC.

Patients and methods

Procedures. This prospective observational study was conducted at a single institute to investigate PTD followed by AC as a preoperative chemotherapy in patients with HER2-positive breast cancer and intended to estimate tumor reduction after PTD alone or after both PTD and AC. The planned study period was from April 2019 to in July 2022, and the registration period was from March 2019 to July 2021. Key inclusion criteria are: HER2-positive breast cancer diagnosed pathologically HER2 3+ by immunohistochemistry or positive results on in situ hybridization if HER2 2+ by immunohistochemistry; patients indicated to receive neoadjuvant chemotherapy; an Eastern Cooperative Oncology Group Performance Status of 0 or 1. Key exclusion criteria were patients with other active cancers that required treatment, those with heart failure (EF <60%), and those whose inclusion was determined to be inappropriate by the investigators. Magnetic resonance imaging (MRI) and ultrasound (US) were used to assess the maximum tumor diameter for breast lesions at baseline, after PTD, and after both PTD and AC; the maximum tumor diameter was measured in accordance with RECIST ver.1.1. If cancer lesions on images were not solitary tumors, the maximum diameter of the area including all lesions was defined as a viable lesion. Contrast-enhanced computed tomography and ultrasound were used to assess the shape and short axis diameter of the axillary lymph node (AxLN).

To assess the primary breast tumor, MRI was evaluated with frequency-selective fat suppression (e-THRIVE) and maximum intensity projection imaging, and the contrast effect range was judged as a viable lesion (Ingenia 3.0T Omega HP release 5.41, Philips Healthcare). Ultrasound was performed by laboratory technicians, who reported the imaging data in clinical documents using Aplio 700, Aplio a550 (Canon) and LOGIQ E9 (GE Healthcare Japan). If a low echoic area with no blood flow was observed with apparent discrepancies compared with MRI images, MRI findings were preferred over US findings. Pathological findings included the diameter of remnant lesions in the surgical pathology report prepared by pathologists, who were blinded to the patient information in this study.

The primary endpoint was the pCR rate after PTD followed by AC, which was defined as ypT0/is and ypN0. Secondary endpoints were tumor reduction rate and clinical complete response (cCR) during PTD treatment, AC treatment, and the sequential full regimen (PTD-AC), and safety, especially cardiotoxicity, at 1 year after surgery. Left ventricular ejection fraction (LVEF) was measured by echocardiography, and the same methods were used for each individual patient throughout the study (7-12). LVEF assessments were performed at baseline and at 1 year after initiation of the planned treatment. Significant declines in the LVEF were defined as decreases over 10% from baseline and to <50% (7-10). Left ventricular systolic dysfunction (LVSD) was reported as a serious adverse event (SAE) (7).

The study protocol was approved by the Clinical Trial Center of Sapporo Medical University, Japan (approval no. 302-237), was conducted in accordance with The Declaration of Helsinki and the Ethical Principles for Medical Research Involving Human Subjects, and is registered with UMIN-CTR (UMIN000046338). We complied with the latest editions of the 'Declaration of Helsinki of the World Medical Association' and the 'Ethical Guidelines for Medical Research Involving Human Subjects' (Ministry of Health, Labour and Welfare) with which all medical research involving human subjects must comply. The consent documents approved by the review committee were given to the subjects (approval no. 302-237), and consent was obtained in writing of the subject's own free will after sufficient explanation.

Treatment protocol. As for the regimen protocol of PTD, patients were given intravenous Pmab on day 1 for three weeks of every cycle; the starting loading dose was 840 mg, which decreased to 420 mg in subsequent cycles. Then, patients were administered Tmab on day 1 of every cycle at a dose of 8 mg/kg, which decreased to 6 mg/kg. Finally, patients were administered docetaxel on day 1 of every cycle at a dose of 75 mg/m^2 after which they were given 6.6 mg of dexamethasone and 0.75 mg of palonosetron. Those drugs were given to patients for four cycles.

As for the subsequent four cycles of AC, patients were given 60 mg/m^2 of doxorubicin and 600 mg/m^2 of cyclophosphamide on day 1 for three weeks of every cycle. Aprepitant at 125 mg was also given intravenously on day 1 and a decreased dose of 80 mg was given orally on days 2 and 3; 1 mg of granisetron was also given intravenously on day 1.

Surgery and perioperative systemic treatments. If the AxLNs were clinically negative and this was discovered before the preoperative treatment, sentinel lymph-node biopsy (SLNB) was performed, while axillary lymph-node dissection (ALND) was performed in cases of clinically positive AxLNs in patients who underwent either mastectomy or partial mastectomy. After surgery, tri-weekly Pmab and Tmab were administered to each patient. During the study period, 1-year trastuzumab emtansine (T-DM1) as a postoperative treatment was covered

by insurance in Japan in accordance with the KATHERINE trial (13). If partial mastectomy was performed, radiation therapy was given to the preserved breast.

Statistical analysis. Descriptive statistics were used to describe the baseline characteristics. Mann-Whitney U test was performed for the reduction rate of PTD, AC, and PTD-AC in those with and without ER positivity. Odds ratios and 95% confidence intervals for pCR for each clinicopathologic factor were calculated by nominal logistic analysis. All statistical analyses were performed using JMP 15.1.0 (SAS Institute Inc., Cary NC, USA).

Results

Patients' background. In total, 25 eligible patients were registered, but one patient declined surgery and was thus ineligible. Therefore, this analysis included 24 patients who were administered PTD-AC for NAC. One patient underwent surgery after 4 cycles of PTD with no AC because of a severe cardiac AE. The baseline patient characteristics are summarized in Table I. The median age of patients was 54.5 years (range, 39-76 years). All patients were diagnosed with invasive breast cancer through core needle biopsy or vacuum-assisted biopsy. As for histological type, patients were diagnosed with two special types, mucinous and invasive lobular carcinomas, in addition to invasive carcinoma of no special type. HER2 overexpression was observed in all patients, and ER and/or PgR positivity was observed in 15 patients. After neoadjuvant chemotherapy, 5 patients underwent partial mastectomy and 19 patients underwent mastectomy. Twelve patients with clinically negative AxLNs underwent SLNB, and of these, one also underwent ALND because of positive SLNB. Another 12 patients underwent ALND alone.

Adjuvant therapy was indicated in all patients; one year of Pmab + Tmab was given to 22 patients and one year of T-DM1 was given to 2 patients, which was available at the hospital as a postoperative treatment. Thereafter, irradiation was indicated after adjuvant therapy in eight patients and was intended for the preserved breast after partial mastectomy or was intended as post-mastectomy radiotherapy (PMRT).

Overall, 23 of 24 patients (%) achieved 85% or more relative dose intensity (RDI) of the planned eight treatment cycles. Four patients had dose reductions but maintained RDI above 85%, while the other 19 patients achieved 100% RDI without dose reductions or discontinuation.

Clinical and pathologic complete response of primary tumors and axillary lymph nodes to NAC. Fig. 1 is sorted by PTD reduction rate and demonstrates the reduction rate of PTD alone and subsequent AC as well as the corresponding pathologic findings. Out of 24 patients, 14 (58.3%) achieved pCR. Of the five patients who underwent partial mastectomy, no case had positive surgical margins. In one patient (#20), AC was omitted and surgery was accelerated because of severe mitral valve disorder after four cycles of PTD, which also resulted in pCR that was evident in the surgical pathology findings. Therefore, 4 of 13 patients (30.8%) achieved pCR after the full regimen (both PTD and AC) and simultaneously achieved cCR by imaging assessment after PTD, while 4 of 14 patients (28.6%) in the intention-to-treat population achieved pCR. The remaining 9 of 14 patients (64.3%) achieved pCR but not cCR at some point after PTD; in other words, they achieved pCR according to their surgical pathology findings with subsequent AC. AxLN metastasis behaved differently from the primary breast tumor (PBT) in some cases, and specifically, there were 5 cases, all of which were ER-positive in which AxLN metastasis ultimately remained. As shown in Fig. 1, the PBTs disappeared in two cases, but AxLN metastases remained (#12 and #19). Table II shows the details of the lymph nodes corresponding to the shrinkage of the PBT. In the remaining three cases (#4, #5, and #9) both the PBT and the AxLN metastases remained. Table III shows the breakdown of pathologic treatment effects. A total of 14 cases achieved pCR as defined in this study: 12 cases of ypT0ypN0 and 2 cases of ypTisypN0.

Table I. Baseline patients' characteristics (n=24).

Characteristic	Values
Median age, years (range)	54.5 (39-76)
cT, 1/2/≥3	4/17/3
cN, 0/≥1	12/12
ER, positive/negative	15/9
PgR, positive/negative	12/12
HER2, 3+/2+ and ISH-positive	23/1
NG, 1-2/3	18/6
Median Ki67, % (range)	32 (14-90)
Breast surgery, mastectomy/partial mastectomy	19/5
Axillary surgery, SLNB/ALND after SLNB/ALND	11/1/12
Adjuvant therapy, 1 year of Pmab + Tmab/1 y of T-DM1	22/2
Radiotherapy, yes/no	8/16

ER, estrogen receptor; PgR, progesterone receptor; NG, nuclear grade; SLNB, sentinel lymph node biopsy; ALND, axillary lymph node dissection; Pmab, pertuzumab; Tmab, trastuzumab; T-DM1, trastuzumab emtansine.

Reduction rate of primary tumors by PTD or AC. The median tumor reduction rate after 4 cycles of PTD was 58.9% (20.8-100%) in all 24 patients, 58.3% (20.8-100%) in patients with ER-positive breast cancer, and 67.6% (31.3-100%) in patients with ER-negative breast cancer (P=0.4733; Fig. 1 and Table II). The median tumor reduction rate after AC was 7.4% (0-79.2%) in 23 patients, 3.9% (0-79.2%) in those with ER-positive breast cancer, and 18.0% (0-68.8%) in those with ER-negative breast cancer (P=0.2915). The overall median tumor reduction rate of total PTD-AC was 76.9% (31.1-100%) in all patients; 73.1% (31.3-100%) in patients with ER-positive breast cancer, and 100% (53.7-100%) in patients with ER-negative breast cancer (P=0.2579).

Univariate analyses for pCR. As shown in Table IV, no statistically significant clinicopathologic factor for pCR was found in the univariate analysis; cT2≤ vs. cT1, OR=2.33,

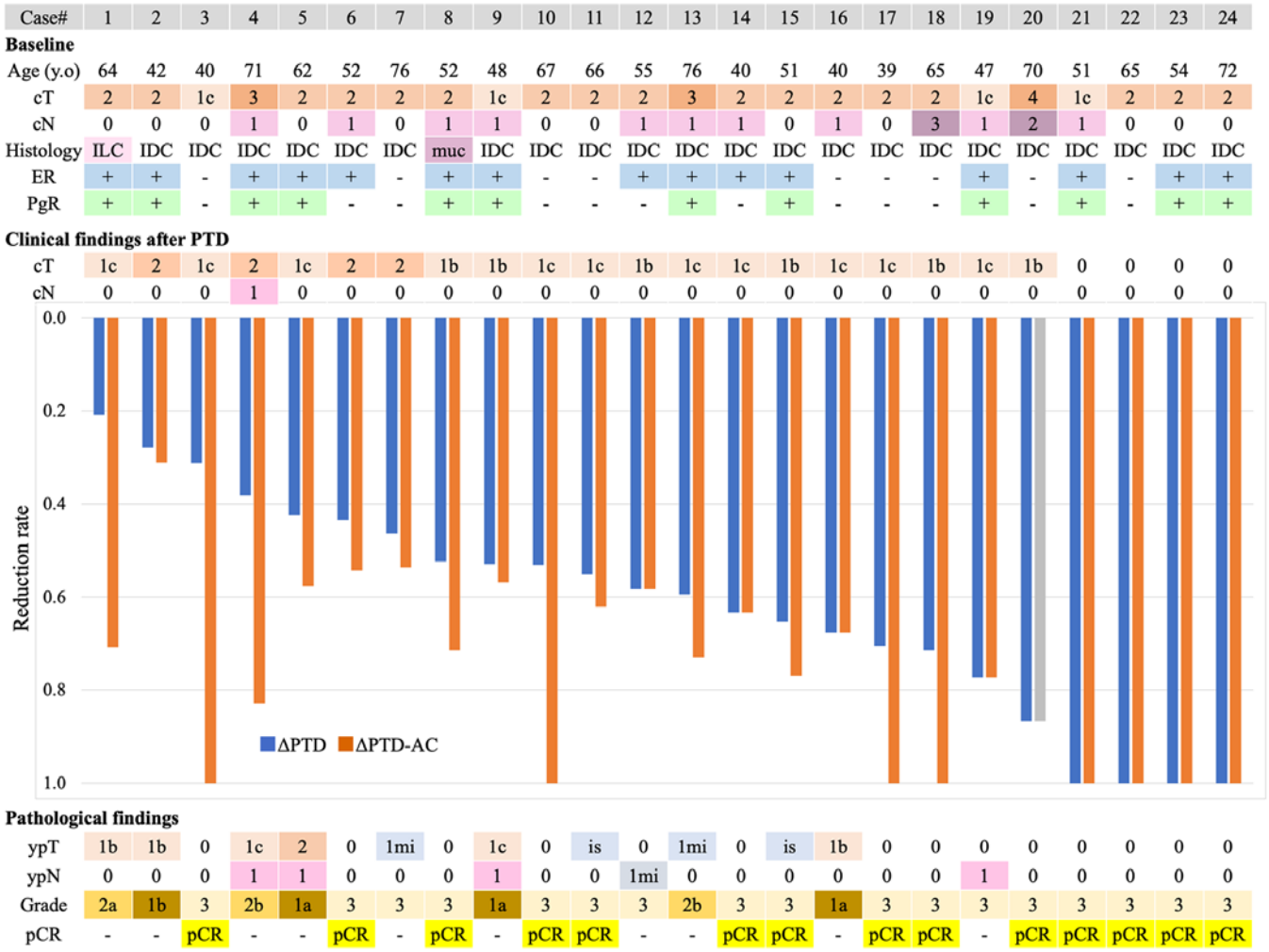

Figure 1. Waterfall plot sorted by PTD reduction rate and clinical and pathological findings during NAC. cCR was observed in 8 of 24 patients with primary breast tumors after sequential PTD-AC, while pCR was observed in 14 of 24 patients. Notably, four patients achieved apparent cCR, and all four patients achieved pCR after PTD alone. IDC, invasive ductal carcinoma; ER, estrogen receptor; PgR, progesterone receptor; NAC, neoadjuvant chemotherapy; cCR, clinical complete response; pCR, pathological complete response; AC, adriamycin and cyclophosphamide; PTD, pertuzumab, trastuzumab and docetaxel; ILC, invasive lobular carcinoma; muc, mucinous carcinoma.

95% CI [0.24-23.57], P=0.4491; cT1≤ vs. cT0 OR=1.00, 95% CI [0.18-5.63], P=1.000; ER-negative vs. ER-positive, OR=2.33, 95% CI [0.39-19.44], P=0.3630; PgR-negative vs. PgR-positive, OR=5.00, 95% CI [0.84-42.54], P=0.0781; Ki67 ≤20% vs. >20% OR=4.00, 95% CI [0.27-109.96], P=0.3115. The presence of 0 items indicated that it was inappropriate to perform a univariate analysis for nuclear grade.

Postoperative relapse events. We conducted an exploratory study in which we analyzed recurrent events. We found that there were no recurrent events over a median follow-up of 22.8 (6.8-37.2) months. The selected adjuvant therapy is described below. Two patients were planned to receive 14 cycles of adjuvant T-DM1, 21 patients were to receive 14 cycles of Tmab + Pmab, and 1 patient was to receive 14 cycles of Tmab. However, the planned adjuvant treatment could not be completed because two patients receiving Tmab + Pmab and one patient receiving Tmab discontinued the treatment because of cardiotoxicity.

Safety. The incidence of treatment-related adverse events (AEs) is summarized in Table V. Alopecia was the most common grade 1-2 AE (n=24), followed by nausea (n=13), palmar-plantar erythrodysesthesia syndrome (n=12), and diarrhea (n=10). Neutropenia or febrile neutropenia was the most common grade 3/4 severe AEs.

As for cardiac safety, in this study, the median observation period for the evaluation of cardiac function (by echocardiography) was 22.0 (range, 3.0-34.3) months. Severe AEs were observed in three cases described below (3/24; 12.5%). One patient had severe mitral regurgitation (grade 3) without significant LVEF reduction two months after the day neoadjuvant chemotherapy (NAC) was initiated, and accordingly, she did not receive anthracycline-containing regimens (#10). Two patients had significant LVEF reductions of 10% or more but <50% from baseline, and they also had symptomatic LVSD (grade 3) at 9.9 and 11.5 months after starting NAC. Both patients experienced LVSD during adjuvant systemic therapy (Tmab + Pmab); the former required intensive care and had a moderate outcome, and the latter also had a moderate outcome but received only oral medication. Those three patients discontinued the planned treatment.

Table II. Clinical and pathological findings on axillary lymph nodes during NAC.

Clinical and pathological findings	Case no.																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Primary breast tumor																								
cT of main tumor baseline	2	2	1c	3	2	2	2	2	1c	2	2	2	3	2	2	2	2	2	1c	4	1c	2	2	2
cT of main tumor after PTD	1c	2	1c	2	1c	2	2	1b	1b	1c	1c	1b	1c	1c	1b	1c	1c	1b	1c	1b	0	0	0	0
ypT	1b	1b	0	1c	2	0	1mi	0	1c	0	is	0	1mi	0	is	1b	0	0	0	0	0	0	0	0
Main tumor reduction rate PTD	0.21	0.28	0.31	0.38	0.42	0.44	0.46	0.52	0.53	0.53	0.55	0.58	0.6	0.63	0.65	0.68	0.71	0.71	0.77	0.87	1	1	1	1
Main tumor reduction rate PTD-AC	1	0.31	1	0.83	0.58	0.54	0.54	0.71	0.57	1	0.62	0.58	0.73	0.63	0.77	0.68	1	1	0.77	Skip	1	1	1	1
Clinical findings of AxLN																								
Swelling AxLN number	0	0	0	2	0	1	0	1	2	0	0	1	1	4	0	5	0	6	1	1	1	0	0	0
AxLN FNA	-	-	-	m	-	m	-	m	m	-	-	m	m	‡	-	m	-	†	m	m	m	-	-	-
cN baseline	0	0	0	1	0	1	0	1	1	0	0	1	1	1	0	1	0	3	1	2	1	0	0	0
cN after PTD	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Short diameter of the largest AxLN																								
Minor axis of AxLN before NAC, mm	*	*	*	37	*	*	*	15	*	*	*	*	*	*	*	20	*	23	*	*	*	*	*	*
Minor axis of AxLN after PTD, mm	*	*	*	32	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Minor axis of AxLN after PTD-AC, mm	*	*	*	15	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Pathological findings of AxLN																								
Results of SLNB for cN0																								
SLNB positive node number	0	0	0	-	1	-	0	-	-	0	0	-	-	-	0	-	0	-	-	-	-	0	0	0
SLNB total number	2	1	1	-	1	-	1	-	-	2	1	-	-	-	1	-	1	-	-	-	-	1	1	2
Results of final LN status including ALND																								
Pathological positive AxLN number	-	-	-	1	3	0	-	0	3	-	-	1	0	0	-	0	-	0	2	0	0	-	-	-
ypN	0	0	0	1	1	0	0	0	1	0	0	1mi	0	0	0	0	0	0	1	0	0	0	0	0

Details of lymph-node metastasis and treatment effects corresponding to the above cases. After PTD-AC, a remnant AxLN metastatic nest was observed in 5 of 12 cN-positive patients, and in all of these patients, the short axis diameter of AxLNs was less than 15 mm, and there were no findings suggestive of malignancy, such as focal thickening of the cortex or disappearance of the lymph-node hilum. Moreover, breast tumors in two patients disappeared, but residual metastases remained in the AxLN, which resulted in non-pCR. cN positivity in 12 patients was diagnosed follows: 10 patients by FNA; 1 who did not undergo FNA but had a clinically apparent metastatic node because of large swelling in 6 AxLNs; and 1 patient had insufficient tissue but had a clinically apparent metastatic node because of swelling in four LNs. Tis, ductal carcinoma in situ; T1mi, micro metastasis; AxLN, axillary lymph node; AxLN, axillary lymph node; FNA, fine needle aspiration; NAC, neoadjuvant chemotherapy; SLNB, sentinel lymph node biopsy; ALND, axillary lymph node dissection; skip, AC was skipped in case #20 because of severe adverse event. -, No metastasis; m: metastasis; †, No FNA but clinically apparent metastatic node. ‡, Insufficient specimens but clinically apparent metastatic node because of many swelling LNs. *, Short diameter of 15 mm or less and no abnormalities on imaging.

Table III. Breakdown of pathologic findings.

Pathological findings	ypT	ypN	Number (n=24)
pCR	ypT0	ypN0	12
	ypTis	ypN0	2
non-pCR	ypT1mi	ypN0	2
	ypT1	ypN0	3
	ypT2	ypN0	0
	ypT0	ypN1mi	1
	ypT0	ypN1	1
	ypTis	ypN1	0
	ypT1mi	ypN1	0
	ypT1	ypN1	2
	ypT2	ypN1	1

There were 14 cases of pCR as defined in this study, 12 cases of ypT0ypN0, and 2 cases of ypTisypN0. In two cases, the PBT disappeared, but AxLN metastases remained. pCR, pathological complete response; PBT, primary breast tumor; AxLN, axillary lymph node.

Table IV. Univariate analysis for pCR.

Clinicopathological factors	Odds ratio	95% CI	P-value
cT (≥cT2 vs. cT1)	2.33	0.24-23.57	0.4491
cN (≥cN1 vs. cN0)	1.00	0.18-5.63	1.0000
ER (negative vs. positive)	2.33	0.39-19.44	0.3630
PgR (negative vs. positive)	5.00	0.84-42.54	0.0781
NG (3 vs. 1-2)	-	-	-
Ki67 (≥20% vs. <20%)	4.00	0.27-109.96	0.3115

pCR, pathological complete response; 95% CI, 95% confidence interval; ER, estrogen receptor; PgR, progesterone receptor; NG, nuclear grade.

Discussion

This study was conducted as a single-center prospective observational study with a PTD-first sequence. We investigated the effect of PTD alone and the additional effects of anthracycline-containing regimens. As for perioperative systemic therapy, regimens preceded by anthracyclines have become the standard of care, and although the order makes no significant difference in safety and efficacy (4,5) in patients with HER2-negative breast cancer, it was not ascertained whether taxane-first sequence would be similarly positioned in patients with HER2-positive breast cancer. The omission of anthracycline-containing regimens has been discussed, particularly to prevent cardiotoxicity during treatment. Simultaneously, the benefits and conditions of adding anthracycline remain unclear. Additionally, no conclusion has been reached, about whether a taxane-first sequence leads to a benefit. pCR in HER2-positive breast cancer is associated with substantial improvement in event-free survival and OS, and therefore, pCR was confirmed as a prognostic surrogate marker in HER2-positive breast cancer (14). Therefore, it would be sensible to analyze the effect of PTD alone and the subsequent additional effect of AC in patients who achieve cCR/non-cCR, if used as a surrogate marker.

In this study, 14 patients in the intention-to-treat population achieved pCR at a rate of 58.3%. There were four cases of cCR with PTD alone, which resulted in pCR at surgery. In contrast, there were also cases of pCR due to AC after non-cCR (9/14 patients; 64.3%), which indicated the possibility of an additional effect of AC administered later. In the NeoSphere study, pCR was defined as the absence of invasive neoplastic cells on microscopic examination of the PBT at surgery, and in that study, the pCR rate of the PTD group was 45.8% (2). Therefore, the result was numerically favorable and even accounted for anthracycline-containing regimens that followed. However, it is important to note that pCR and cCR differ in quality, discussed below. In the GeparQuattro study, compared the efficacy of the HER2 overexpression status in patients with breast cancer in the NAC setting, which four cycles of docetaxel or docetaxel plus capecitabine were administered preoperatively after 4 cycles of EC (15). In the HER2-positive group, the cCR rate after four cycles of EC was 22.0% (98/445), and after subsequent administration of taxane, the pCR rate was 31.7% (141/445). It is possible to interpret that anthracycline has a certain effect on HER2-positive breast cancer. Recently, antibody-drug conjugates, such as trastuzumab deruxtecan (16), for HER2-positive breast cancer have been introduced sequentially with very impactful results, and clinical trials focusing on therapies other than anti-HER2 therapy have become rare except for those testing carboplatin. In this sense, the data are valuable and show that anthracycline-containing regimens lead to at least a certain level of response. Since the results of this study also showed that some cases, which had non-cCR after PTD and pCR after the entire regimen, responded to anthracycline, it might be better not to omit anthracycline so readily but to carefully examine the cases in which the omission can be considered.

In the NAC setting, trials omitting anthracycline have been reported, although most protocols use carboplatin instead of anthracycline. In the TRAIN-2 study, the pCR rate was similar [the difference was -1.5% between the anthracycline and the non-anthracycline groups; 95% CI (-11-8), P=0.95 (9)], and no substantial difference was observed in cardiac toxicity with or without anthracyclines (10). Notably, the results are similar regardless of whether the cancer was ER-positive or ER-negative (9). The BCIRG-006 trial also reported similar data on DFS and cardiac toxicity, albeit in an adjuvant setting (12). Therefore, anti-HER2 therapy using docetaxel and carboplatin as the chemotherapy components is a reasonable approach for stage II or III HER2-positive tumors in either the adjuvant or neoadjuvant setting (17). For anatomic stage I (T1N0), HER2-positive tumors, paclitaxel plus trastuzumab is a good de-escalating option according to the APT trial (17-19). However, the use of carboplatin differs significantly from taxane-based regimens in that it has high treatment intensity and a high incidence of side effects (20). Few trials have examined the omission of anthracycline as a taxane base, but one searchable retrospective study

Table V. Summary of adverse events in patients.

Adverse event	Total	Grade 1-2	Grade ≥3
Blood and lymphatic system disorders			
Neutropenia	4	2	2
Anemia	1	1	0
Febrile neutropenia	3	-	3
Cardiac disorders			
Left ventricular systolic disfunction	2[a]	-	2
Mitral valve disorder	1	0	1
General disorders and administration site conditions			
Fatigue	8	8	0
Peripheral edema	6	6	0
Mucosal inflammation	0	0	0
Fever	2	2	0
Skin and subcutaneous tissue disorders			
Alopecia	24	24	0
Rash	9	9	0
Nail disorder	3	3	0
Pruritus	2	2	0
Dry skin	1	1	0
Skin hyperpigmentation	1	1	0
Palmer-plantar erythrodysesthesia syndrome	12	12	0
Pruritus	2	2	0
Gastrointestinal disorders			
Diarrhea	10	10	0
Nausea	13	13	0
Vomiting	1	1	0
Constipation	2	2	0
Abdominal pain	1	1	0
Mucositis oral	2	2	0
Metabolism and nutrition disorders			
Anorexia	2	2	0
Nervous system disorders			
Headache	2	2	0
Peripheral motor neuropathy	1	1	0
Peripheral sensory neuropathy	4	4	0
Dysgeusia	3	3	0
Musculoskeletal and connective tissue disorders			
Myalgia	2	2	0
Arthralgia	2	2	0
Respiratory, thoracic and mediastinal disorders			
Cough	2	2	0
Other adverse events			
Cystitis noninfective	1	1	0
Shingles	2	2	0
Middle ear inflammation	1	1	0

[a]One patient with LVEF reduction over 10% was non-symptomatic.

was found; that study used propensity score matching and reported a similar 3-year progression-free survival without anthracycline (20). Therefore, further analysis of anthracycline omission or evidence-based use is necessary.

Pivotal studies on the prognostic improvement effect of adding Pmab or other antiHER2 drugs, in the treatment regimen have been reported. The combination of Tmab with Pmab improves invasive disease-free survival (IDFS) in adjuvant chemotherapy for HER2-positive breast cancer (21). In the NAC setting, the addition of Pmab to preoperative chemotherapy improved the pCR rate, but did not directly improved prognosis (2,11). However, in patients with HER2-positive breast cancer, the prognostic value of pCR has been clarified in a study (14). Thus, further prognostic analysis may reveal an improving effect with the use of antiHER2 drugs, in the near future. The addition of T-DM1 for 1 year to non-pCR in preoperative chemotherapy for patients with HER2-positive breast cancer reduced 3yIDFS events from 22.2 to 12.2% compared with the no addition group. However, for OS, the HR 0.70 with 95% CI of 0.47-1.05, which was not significant, but the absolute value was reduced from 7.5 to 5.7% (22). In the KATHERINE trial, the combination of Tmab + Pmab as antiHER2 therapy was administered in 18% of patients (22). In the present study, we analyzed recurrent events after surgery as an exploratory investigation over a median follow-up of 22.8 months, and there were no recurrent events observed during this period. Therefore, the difference between cCR and non-cCR after PTD or that between pCR and non-pCR after PTD-AC could not be clarified in this study. However, it is worth noting that there were no recurrences after these treatments during the postoperative period of approximately 2 year.

A meta-analysis of the diagnostic performance of MRI in HER2-positive breast cancer received NAC revealed a positive predictive value (PPV) of 62.0-94.6% for non-pCR (residual disease) diagnostic, a negative predictive value (NPV) of 34.9-72%, and sensitivity of 47-90% (23). However, in the present study, the PPV for the presence of residual tumor was 62.5% (10/16), the sensitivity was 100% (10/10), and the false-negative rate was 0% (0/10). Compared with the meta-analysis, the PPV was relatively low, which was a limitation of the present study, although the high sensitivity and low false-negatives rates appeared favorable.

According to the combination of US, there was no difference between MRI and US accuracy [P=0.15, (24)]. The accuracy of diagnosing the presence of residual disease was reported with a PPV of 92.1% and NPV of 51.0% for MRI alone, whereas for MRI and US, the PPV was 94.0% and the NPV was 57.7%, suggesting that the combination of MRI and US is superior in detecting residual disease but has problems in ruling out residual disease (25,26).

Although the limitations in detecting residual lesions due to PTD, should be noted, diagnoses made by MRI/US are performed with a certain degree of accuracy in daily practice. Recently, pCR/NAC diagnosis using core needle biopsy or vacuum-assisted biopsy improves NPV and resulted in a favorable false-negative rate (27,28); accordingly, pCR/NAC has become a considerable option. Unfortunately, we did not perform post-PTD biopsy in this study. Thus, it is one of the limitations as described later in this study. However, it is worth noting that in some cases of non-cCR after PTD, AC can still have an additional effect and become pCR.

Regarding cardiac disorders, the cardiotoxicity of anthracycline administration in addition to that of anti-HER2 therapy is a problem that should not be underestimated. In this study, AEs other than cardiotoxicity did not differ significantly from those previously reported. However, LVSD was observed in two patients, and one patient experienced severe mitral regurgitation but no significant LVEF reduction. The results showed a higher frequency of LVSD events of any grade (3/24; 12.5%) than TRYPHAENA (7). Evidence for a taxane-first sequence is not sufficient (4,5). In this study, the pCR rate was favorable, and as for safety, the frequency of cardiac complications was slightly higher.

A limitation of this prospective observational study is the small number of cases, because of the low frequency of HER2-positive rates, 10-20% of all breast cancers (29). The second limitation is the lack of sufficient statistical studies because of the small case number. However, considering past reports, even with a small number of cases, issues that were later considered had initially materialized from observational studies including this study. Third, problems may arise in MRI/US assessment, where diagnostic imaging is a different technique than pathology. It is therefore not possible to make a diagnosis of exactly the same quality, for example, the diagnosis of residual lesions or complete tumor disappearance before starting additional AC. In future investigation, it may be important to perform a minimally invasive biopsy in addition to diagnostic imaging, which might at least improve the reliability of the diagnosis of residual disease, and might be supportive to investigate alternative treatment options based on the diagnosis of residual disease after prior neoadjuvant systemic therapy; for example, a study to investigate the benefit of adding AC for patients with residual lesions after PTD might be more convincing.

In conclusion, we again found that the pCR rate and response rate to PTD were high but that some cases experienced an additional effect of AC, which resulted in pCR. As cardiotoxicity remains a significant problem, further research on the risk-benefit treatment strategy is needed to target the omission of AC or for selection of patients expecting an additional AC effect. For taxane-first sequences, a high pCR rate was observed, although it should be noted that the frequency of cardiac AEs could not be ignored.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HS, GK, DK, HK, TO and IT conceived and planned in detail the present study. YK, FS, NN and TH extracted the entirety of patient data and performed the data construction and put the

data into a form that could be entered into statistical software. AW, KS and SU performed analysis and interpretation of the patient data with HS. DK, HK and TO revised it critically for important intellectual content. IT provided overall supervision and gave final approval to the publishable version. All authors read and approved the final manuscript. HS and GK confirm the authenticity of all the raw data.

Ethics approval and consent to participate

This study adhered to ethical tenets of The Declaration of Helsinki and Ethical Principles for Medical Research Involving Human Subjects, was approved by the Clinical Trial Center of Sapporo Medical University, Japan, and is registered with UMIN-CTR (UMIN000046338). The consent documents approved by the review committee were given to the subjects (302-237), and consent was obtained in writing of the subject's own free will after sufficient explanation.

Patient consent for publication

Patients provided written informed consent for publication.

Competing interests

The authors declare that they have no competing interests.

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