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PHASE I STUDIES

Phase 1 combination study of Eribulin mesylate with trastuzumab for advanced or recurrent human epidermal growth factor receptor 2 positive breast cancer

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Summary Eribulin mesylate (Halaven®) is a novel inhibitor of microtubule dynamics that has demonstrated a survival benefit in patients with locally recurrent or metastatic breast cancer who previously received at least two chemotherapeutic regimens including an anthracycline and a taxane. Although trastuzumab is indicated for patients with human epidermal growth factor receptor 2 positive (HER2+) breast cancer, a phase 1 study to evaluate tolerability/safety of eribulin mesylate with trastuzumab has not been conducted. Therefore, a study of eribulin mesylate in combination with trastuzumab was conducted to evaluate dose limiting toxicity (DLT), tolerability/safety, pharmacokinetics (PK), and efficacy and to estimate the recommended dose in Japanese patients with advanced or recurrent HER2+ breast cancer. Eribulin mesylate (1.4 mg/m^2) was administered on days 1 and 8 of every 3 week cycle. Trastuzumab was administered with a 4 mg/kg loading dose followed by 2 mg/kg weekly doses or with an 8 mg/kg loading dose followed by 6 mg/kg tri-weekly doses. A total of 12 patients (six for each regimen) received eribulin mesylate and trastuzumab. No DLT was observed and the recommended dose of eribulin mesylate in combination with trastuzumab was estimated as 1.4 mg/m^2. Common adverse events were neutropenia, leukopenia, anaemia and alopecia. This combination therapy was well tolerated and the neutropenia observed was manageable. No PK drug-drug interaction between eribulin and trastuzumab was observed. Since a transient ejection fraction decreased was observed in two patients, cardiac function should be routinely assessed in patients receiving the combination therapy of eribulin mesylate with trastuzumab (ClinicalTrials.gov Identifier: NCT01432886).

Keywords Eribulin mesylate · HER2 positive breast cancer · Japanese patients · Phase 1 study · Trastuzumab

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Introduction

Overexpression of human epidermal growth factor receptor 2 (HER2) occurs in approximately 25 to 30 % of breast cancers and is associated with a poor prognosis [1, 2]. Trastuzumab is a humanized IgG1 kappa monoclonal antibody that selectively binds with high affinity to the extracellular domain of the HER2 protein. The studies of weekly trastuzumab monotherapy (4 mg/kg loading dose followed by 2 mg/kg weekly) and combination with paclitaxel was active, well tolerated and prolonged survival in patients with HER2+ metastatic breast cancer [3–5]. Also,

comparison of tri-weekly trastuzumab (8 mg/kg loading dose followed by 6 mg/kg tri-weekly dose) with weekly trastuzumab has shown comparable results in both monotherapy and combination therapy with paclitaxel [6, 7]. The combination therapy of a tubulin-targeted drug and trastuzumab appeared to have a superior antitumor effect and a well-tolerated safety profile in the treatment of HER2 + breast cancer [5, 7–9].

Eribulin mesylate, a non-taxane microtubule dynamics inhibitor, is a structurally simplified synthetic analog of the marine natural product halichondrin B. The inhibitory effects of eribulin on microtubule dynamics lead to G_2/M cell-cycle blocks, disruption of normal mitotic spindle formation, and prolonged mitotic blockage followed by apoptotic cell death [10, 11].

A phase 1 study has been completed in Japanese patients with solid tumors to evaluate the safety and pharmacokinetics (PK) of eribulin mesylate administration on days 1 and 8 of a 21-day cycle. Dose-limiting toxicities (DLTs) were neutropenia/febrile neutropenia, and the recommended dose of eribulin mesylate was determined as 1.4 mg/m^2 [12]. Based on the results of this phase 1 study in Japan, a phase 2 study was conducted to evaluate the efficacy and safety of eribulin mesylate in patients with locally advanced or metastatic breast cancer previously treated with anthracycline and taxane. Eribulin mesylate demonstrated antitumor activity with an objective response rate (ORR) of 21.3 % (17/80 patients) and a manageable safety profile [13]. This study supported the previous phase 2 study of eribulin mesylate that demonstrated its antitumor activity and safety profile in extensively pretreated breast cancer patients [14, 15].

In a randomized phase 3 study of patients with locally recurrent or metastatic breast cancer who previously received at least two chemotherapeutic regimens including an anthracycline and a taxane, the efficacy and safety of eribulin mesylate (1.4 mg/m^2, days 1 and 8 of a 21-day cycle) were compared with the treatment of the physician's choice (TPC). Overall survival (OS) was statistically significantly longer in the eribulin mesylate group than in the TPC group (median OS: 13.1 months vs. 10.6 months, hazard ratio [HR]: 0.81, p=0.041). Furthermore, an updated analysis of OS confirmed the significant increase in OS of the eribulin mesylate group compared with the TPC group (median OS: 13.2 months vs. 10.5 months, HR: 0.81, p= 0.014) [16].

Based on these results, the combination therapy of eribulin mesylate and trastuzumab was also expected to provide a superior antitumor effect and favorable safety profile.

Therefore, a phase 1 study of eribulin mesylate in combination with trastuzumab in Japanese patients with advanced or recurrent HER2+ breast cancer was carried out.

Materials and methods

Study design and treatment

This was a multi-center, open-label phase 1 study of eribulin mesylate with trastuzumab combination therapy in Japanese patients with advanced or recurrent HER2+ breast cancer (NCT01432886). This study consisted of Part 1 (weekly dose of trastuzumab) and Part 2 (tri-weekly dose of trastuzumab) to evaluate DLT, tolerability/safety, efficacy and PK, and to estimate the recommended dose of eribulin mesylate in this combination therapy.

Eribulin mesylate was administered by 2- to 5-minute i.v. injection on days 1 and 8 of a 21-day cycle. The initial dose of eribulin mesylate was 1.4 mg/m^2 (equivalent to 1.23 mg/m^2 eribulin expressed as free base) in combination with trastuzumab treatment. Eribulin mesylate was administered on days 1 and 8, if all of the following criteria were met: (1) neutrophil count $\geq 1.0 \times 10^3/\mu L$, (2) platelet count $\geq 7.5 \times 10^4/\mu L$, and (3) non-hematologic toxicity $\leq$grade 2 (except grade 3 nausea, vomiting, and diarrhea controllable with antiemetic or anti-diarrheal medication and abnormal laboratory parameters not requiring treatment). If the patient did not meet the criteria, the administration of the next dose was delayed. If the patient met the criteria within a 1-week delay, the 2nd administration of the cycle was implemented, and the next cycle had to be initiated no sooner than 2 weeks after the 2nd administration. If the patient did not meet the criteria within a 1-week delay, the 2nd administration was skipped. Dose reduction of eribulin mesylate could be exercised at the discretion of the investigator if the 2nd administration in a cycle was delayed or skipped. If dose reduction was necessary, the 1.4 mg/m^2 dose of eribulin mesylate was initially reduced to 1.1 mg/m^2 and then further reduced to 0.7 mg/m^2.

In Part 1, trastuzumab was administered by i.v. infusion at 4 mg/kg as a loading dose and at 2 mg/kg weekly. In Part 2, trastuzumab was administered by i.v. infusion at 8 mg/kg as a loading dose and at 6 mg/kg tri-weekly. The infusion time of trastuzumab was 90 min or longer at initial administration and could be shortened to 30 min from the 2nd administration and later. Trastuzumab was administered immediately after eribulin mesylate administration when used in the same day. Concomitant use of other medications or treatments was allowed. However, other anti-cancer drugs, investigational drugs and prophylactic administration of granulocyte-colony stimulating factor (G-CSF) were not permitted during the study.

DLT was evaluated in the first cycle, and if DLTs were observed in none or one of the first three patients, an additional three patients were to be added at the same dose level. If none or one of a total of six patients experienced a DLT, the investigated dose level of eribulin mesylate was to be regarded

as tolerable with the trastuzumab combination. In the event that two of six patients reported a DLT, the investigators were to obtain written or verbal advice from an Independent Safety Committee on whether to investigate a decreased dose level of eribulin mesylate (1.1 mg/m^2) as the initial dose. The initial eribulin mesylate dose level was planned to decrease to 1.1 mg/m^2 if a DLT was reported in three or more patients. Patients were to continue to receive eribulin mesylate until they no longer received clinical benefit, had progressive disease (PD), or experienced unacceptable toxicity.

The protocol was approved by the Institutional Review Board and conducted in accordance with the Declaration of Helsinki.

Patient eligibility

Key inclusion criteria included: 20–74 years of age; histologically or cytologically confirmed advanced or recurrent breast cancer; HER2+ tumor score of 3+ by immunohistochemistry staining or gene amplification by fluorescence in situ hybridization (FISH); and any of the following, 1) evidence of recurrence during adjuvant chemotherapy with trastuzumab and taxane, 2) evidence of recurrence within 6 months after adjuvant chemotherapy with trastuzumab and taxane or 3) prior chemotherapy including trastuzumab and taxane for advanced or recurrent breast cancer; normal function in major organs; left ventricular ejection fraction (LVEF) ≥60 % by multigated acquisition scan or echocardiogram (B or M mode); and Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.

Key exclusion criteria included: brain metastasis accompanied by clinical symptoms or requiring treatment; severe active infection requiring treatment; pleural effusions, ascites or pericardial effusions requiring drainage; pregnancy or breastfeeding; and refusal of supportive therapy by blood transfusion. All patients provided written informed consent prior to any study procedure.

Assessments

DLTs

The following toxicities were to be regarded as DLTs if they occurred in the first cycle and their causal relationship with the study treatment could not be ruled out: grade 4 neutropenia persistent for more than 7 days; grade 3 or above febrile neutropenia; grade 4 thrombocytopenia or grade 3 thrombocytopenia requiring blood transfusion; and non-hematologic toxicity ≥grade 3 (unless grade 3 nausea, vomiting and diarrhea was controllable with an anti-emetic and anti-diarrheal medication, or clinical laboratory abnormalities did not require treatment). It would also be regarded as a DLT if the 2nd eribulin mesylate administration per cycle was delayed and the study treatment could not be resumed from day 22 of cycle 1.

Safety

The demographic and disease characteristics of breast cancer were recorded at baseline and included HER2 status, estrogen receptor status, progesterone receptor status, and prior therapy complications. Safety assessments were made throughout the study. The factors that were assessed include adverse events (AEs), vital signs, bodyweight, 12-lead electrocardiogram, multigated acquisition scan or echocardiogram, concomitant medications, and clinical laboratory values (hematology, blood biochemistry and urinalysis). AEs were assessed on days 1, 8 and 15 of each cycle. AE severity was classified according to the Japanese version of Common Terminology Criteria for Adverse Events v4.0. QT interval corrected for heart rate using Fridericia's formula (QTcF) and LVEF assessment were conducted before the treatment, on day 15 of cycle 1, on day 1 of every 4th cycle, and if clinically indicated.

PK and biochemical methodology

Blood samples were taken for eribulin PK analysis on days 1 and 8 of the first treatment cycle, pre-dose, end of infusion, 30 min, and 1, 2, 4, 24, 72 and 168 h after drug administration. Plasma concentrations of eribulin, measured as the free-base (i.e. non-mesylate) equivalent, were determined by using the validated liquid chromatography- tandem mass spectrometry method. The lower limit of quantitation was 0.2 ng/mL.

In Part 1, blood samples were taken for trastuzumab PK analysis on day 1 (pre-dose, end of infusion, and 3, 4, 24 and 72 h after drug administration) and day 8 and 15 (pre-dose) of cycle 1, and on day 1 (pre-dose) of cycle 2 and later. In Part 2, blood samples of day 1 (pre-dose and 3, 4, 24, 72, 168 and 336 h after drug administration) of cycle 1 and day 1 (pre-dose) of cycle 2 and later cycles were taken. Serum concentrations of trastuzumab were determined by using the validated enzyme-linked immunosorbent assay method. The lower limit of quantitation was 10 μg/mL.

PK parameters for eribulin and trastuzumab were calculated by a non-compartmental approach by using WinNonlin software version 6.2 (Pharsight Corporation, CA, USA). The calculated parameters included: area under the curve extrapolated to infinity ($AUC_{(0\text{-inf})}$), terminal half-life ($t_{1/2}$), total clearance (CL) and volume of distribution at steady state (V_{ss}). The maximum observed plasma concentration (C_{max}) and time to C_{max} (t_{max}) were directly derived from the data.

Tumor assessment

Tumor response was assessed by the investigators according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [17]. Tumor assessments were performed within 28 days prior to the start of treatment, 6 weeks after the first dose and every 6 weeks thereafter, or sooner, if there was clinical suspicion of disease progression.

Results

Results are based on data collected until an August 2, 2013, cutoff date.

Patient characteristics

A total of 12 patients received eribulin mesylate and trastuzumab, 6 in Part 1 and 6 in Part 2. These patients were extensively pretreated with a median of 4.5 (range, 1–14) prior chemotherapy regimens. Patient demographics and baseline characteristics are shown in Table 1.

Treatment

For the overall population, eribulin mesylate and trastuzumab were administered for a median of 7 (range, 2–23) cycles, with eight patients receiving five or more cycles. The numbers of treatment cycles by patients are shown in Table 2. Treatment was discontinued in seven patients (58.3 %) due to PD, and two patients (16.7 %) withdrew due to AEs. Three patients (25.0 %) continued to receive the study drug treatment at the time of the cutoff date. Dose adjustment (reduction, delay or skip) of eribulin mesylate occurred in ten patients (83.3 %): eight patients (66.7 %) had dose reduction, eight patients (66.7 %) had dose delay, and five patients (41.7 %) had to skip one dose per cycle.

DLTs

DLTs were evaluated on the first cycle, and none of the 12 patients experienced DLT. In cycle 1, grade 3 or 4 neutropenia that led to a dose delay of the day 8 administration of eribulin mesylate occurred in two patients (16.7 %), and a dose was skipped in two patients (16.7 %). Two patients (16.7 %)

Table 1 Patient demographics and baseline characteristics

Parameter	Part 1 (*N*=6)	Part 2 (*N*=6)	Total (*N*=12)
Age, median (range), years	64.5 (58–72)	47.0 (39–64)	60.0 (39–72)
Race, n (%)			
Japanese	6	6	12 (100.0)
ECOG performance status, n (%)			
0	3	2	5 (41.7)
1	3	4	7 (58.3)
ER + and/or PR + disease, n (%)	2	3	5 (41.7)
Number of prior anti-cancer drug treatment, n (%)[a]			
1	0	1	1 (8.3)
2	0	1	1 (8.3)
3	2	0	2 (16.7)
4	1	1	2 (16.7)
≥5	3	3	6 (50.0)
Median (range)	5.0 (3–14)	4.5 (1–6)	4.5 (1–14)
Prior trastuzumab treatment, n (%)	6	6	12 (100.0)
Number of prior trastuzumab treatment, n (%)[a]			
1	2	1	3 (25.0)
2	1	3	4 (33.3)
≥3	3	2	5 (41.7)
Median (range)	2.5 (1–5)	2.0 (1–4)	2.0 (1–5)
Prior taxane treatment, n (%)	6	6	12 (100.0)
Number of prior taxane treatment, n (%)[a]			
1	3	4	7 (58.3)
2	2	2	4 (33.3)
3	1	0	1 (8.3)
Prior anthracycline treatment, n (%)	3	3	6 (50.0)

ECOG Eastern Cooperative Oncology Group, *ER* estrogen receptor, *PR* progesterone receptor

[a] Including the number of neoadjuvant, adjuvant and therapeutic therapy

Table 2 Numbers of treatment cycles by patients

Number of cycles received, n (%)	Part 1 (*N*=6)	Part 2 (*N*=6)	Total (*N*=12)
1–2		1	1 (8.3)
3–4	2	1	3 (25.0)
5–8		3[a]	3 (25.0)[a]
9–12	2		2 (16.7)
13–16		1[a]	1 (8.3)[a]
17–20			
≥21	2[a]		2 (16.7)[a]

[a] 3 patients in total were on treatment as of 2 August 2013

received concomitant treatment with G-CSF for grade 4 neutropenia. The median time from day 1 of cycle 1 to the nadir in neutrophil count was 15 days (95 % CI:14, 23), and the median time to recovery from the nadir to ≤grade 2 neutropenia was 8 days (95 % CI: 4, 9). One of six patients (16.7 %) skipped a dose of trastuzumab due to an ejection fraction decreased (grade 2) in Part 1 (weekly dose of trastuzumab).

Adverse events

The AEs (all grades) experienced by ≥10 % of patients and the total grade 3 or 4 AEs are shown in Table 3. The common AEs were similar in Part 1 and 2. Frequently observed hematologic AEs were neutropenia [100 % (grade 3/4: 100 %)], leukopenia [100 % (grade 3/4: 83 %)] and anaemia [67 % (grade 3/4: 0 %)]. G-CSF was administered to three patients (25.0 %). Frequently observed non-hematologic AEs, which were generally mild and manageable, included alopecia (67 %), pyrexia (42 %), decreased appetite (42 %) and rash (42 %) (all grade 1 or 2). Peripheral neuropathy occurred in four patients (33.3 %), including one patient (8.3 %) with grade 3 neuropathy. The AEs that led to study discontinuation were peripheral neuropathy in one patient (8.3 %) and tumour pain in one patient (8.3 %). An ejection fraction decreased (grade 2) occurred in two patients (16.7 %) (both patients on day 15 of cycle 1 with one patient also in cycle 19), but the patients recovered after 1 week without treatment. The mean LVEF transition is shown in Fig. 1. The other cardiac disorders were second degree atrioventricular block (grade 2) in one patient (8.3 %) and palpitation (grade 1) in two patients (16.7 %); no treatments were required for these adverse advents. No grade 5 AEs or serious AEs were observed.

PK

Figure 2 shows the mean plasma eribulin concentration time profile up to 168 h and Table 4 shows the PK parameters of eribulin after eribulin mesylate (1.4 mg/m^2) administration over 2 to 5 min with trastuzumab on day 1 of cycle 1 in Part 1 and Part 2, as well as previously reported phase 1 study results of eribulin mesylate monotherapy in Japanese patients [12].

In Part 1, the mean values for $t_{1/2}$, CL and V_{ss} after administration of eribulin mesylate on day 1 (Table 4) and Day 8 were 38.1±7.80 and 30.3±3.29 h, 2.47±0.774 and 2.44±0.967 L/h/m^2, and 101±45.3 and 77.9±37.6 L/ m^2, respectively. In Part 2, the mean values for $t_{1/2}$, CL and V_{ss} after administration of eribulin mesylate on day 1 (Table 4) and Day 8 were 35.0±10.8 and 31.7±8.58 h, 2.12±0.754 and 1.95±0.721 L/h/m^2, and 69.8±11.8 and 58.0±6.99 L/m^2, respectively.

After trastuzumab was administered intravenously in combination with eribulin, trastuzumab was eliminated from the serum biphasically after reaching the C_{max} in both Part 1 and 2. In Part 1, the mean values for $t_{1/2}$, CL and V_{ss} after administration of trastuzumab (4 mg/kg) on day 1 were 115 ±28.0 h, 0.369±0.0297 mL/h/kg and 62.4±17.9 mL/kg, respectively. In Part 2, the mean values of $t_{1/2}$, CL and V_{ss} after administration of trastuzumab (8 mg/kg) on day 1 were 173± 26.7 h, 0.271±0.0343 mL/h/kg and 62.0±4.04 mL/kg, respectively (Table 5).

Antitumor activity

Tumor responses were evaluated by RECIST version 1.1 in all 12 patients. The ORR was 8.3 % (95 % CI: 0.2, 38.5) and tumor responses consisted of a partial response (PR) in one patient (8.3 %), stable disease (SD: including non-complete response (CR)/non-PD) ≥5 weeks) in ten patients (83.3 %) and PD in one patient (8.3 %). The disease control rate (CR + PR + SD ≥11 weeks) was 83.3 % (95 % CI: 51.6, 97.9) and the clinical benefit rate (CR + PR + SD ≥23 weeks) was 50.0 % (95 % CI: 21.1, 78.9) (Table 6).

Discussion

This phase 1 study established the recommended dose of eribulin mesylate as 1.4 mg/m^2 when administered on days 1 and 8 of a 21-day cycle with appropriate dose adjustment in combination with either weekly trastuzumab (4 mg/kg loading dose, 2 mg/kg/weekly) or tri-weekly trastuzumab (8 mg/kg loading dose, 6 mg/kg/tri-weekly) in Japanese patients with advanced or recurrent HER2+ breast cancer. Eribulin mesylate was suggested to be safe and tolerable in combination with trastuzumab with the same recommended dose as monotherapy [12].

There were no DLTs, grade 5 AEs or serious AEs in this study. The most common AEs of grade 3 or 4 reported in this study were neutropenia and leukopenia, which were

Table 3 Adverse events (All grades in ≥10 % of patients and grades 3/4 in total)

	Part 1		Part 2		Total	
	All grades	Grade 3/4 AEs	All grades	Grade 3/4 AEs	All grades	Grade 3/4 AEs
AE preferred term	*N*=6	*N*=6	*N*=6	*N*=6	*N*=12	*N*=12
Blood and lymphatic system disorders						
Neutropenia	6	6	6	6	12 (100.0)	12 (100.0)
Leukopenia	6	4	6	6	12 (100.0)	10 (83.3)
Anaemia	4	0	4	0	8 (66.7)	0
Lymphopenia	0	0	3	3	3 (25.0)	3 (25.0)
Febrile neutropenia	0	0	1	1	1 (8.3)	1 (8.3)
Cardiac disorders						
Palpitations	2	0	0	0	2 (16.7)	0
Gastrointestinal disorders						
Nausea	1	0	2	0	3 (25.0)	0
Vomiting	0	0	3	0	3 (25.0)	0
Constipation	2	0	0	0	2 (16.7)	0
Stomatitis	1	0	1	0	2 (16.7)	0
General disorders and administration site conditions						
Pyrexia	2	0	3	0	5 (41.7)	0
Malaise	3	0	0	0	3 (25.0)	0
Chest discomfort	1	0	1	0	2 (16.7)	0
Injection site reaction	2	0	0	0	2 (16.7)	0
Infections and infestations						
Lung infection	0	0	2	0	2 (16.7)	0
Tonsillitis	1	0	1	0	2 (16.7)	0
Investigations						
Alanine aminotransferase increased	2	0	1	0	3 (25.0)	0
Aspartate aminotransferase increased	2	0	1	0	3 (25.0)	0
Blood creatine phosphokinase increased	3	0	0	0	3 (25.0)	0
Ejection fraction decreased	1	0	1	0	2 (16.7)	0
Metabolism and nutrition disorders						
Decreased appetite	4	0	1	0	5 (41.7)	0
Hypertriglyceridaemia	1	1	2	2	3 (25.0)	3 (25.0)
Hypophosphataemia	0	0	1	1	1 (8.3)	1 (8.3)
Musculoskeletal and connective tissue disorders						
Myalgia	4	0	0	0	4 (33.3)	0
Muscle spasms	2	0	0	0	2 (16.7)	0
Nervous system disorders						
Dysgeusia	2	0	2	0	4 (33.3)	0
[a]Peripheral neuropathy	4	1	0	0	4 (33.3)	1 (8.3)
Headache	0	0	2	0	2 (16.7)	0
Skin and subcutaneous tissue disorders						
Alopecia	5	–	3	–	8 (66.7)	–
Rash	4	0	1	0	5 (41.7)	0

[a] Peripheral neuropathy includes neuropathy peripheral and peripheral sensory neuropathy in preferred terms

hematologic toxicities also found in prior clinical studies of eribulin mesylate monotherapy in Japanese patients [12, 13]. All 12 patients experienced neutropenia, but all events were reversible and easily managed with appropriate dose reduction or a skipped dose. The median time of 8 days required for resolution from nadir in neutrophil count to ≤grade 2 neutropenia in cycle 1 and the low requirement for G-CSF use (25 %) supported the safety of the defined eribulin mesylate

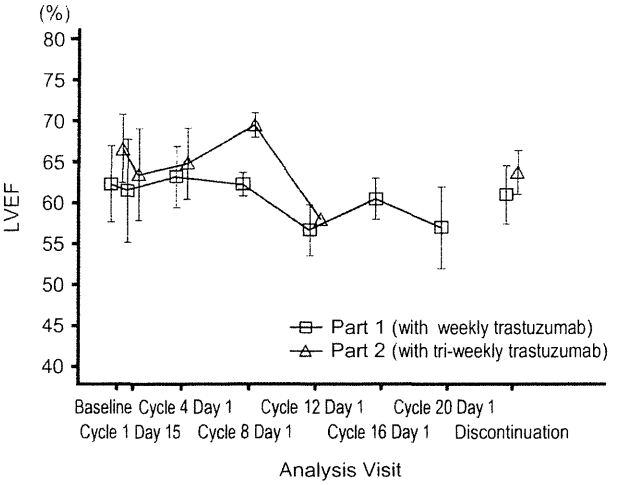

Fig. 1 Mean LVEF transition assessed by echocardiogram: Assessment by B mode echocardiogram at the indicated times. Baseline LVEF was ≥60 % by B or M mode echocardiogram in all patients, as defined in the inclusion criteria

administration procedure. The non-hematologic AEs were consistent with the known safety profile of eribulin, with only one grade 3 or higher AE with clinical symptoms (grade 3 peripheral neuropathy). The low incidence of neuropathy was consistent with previous eribulin mesylate studies and the incidence was also lower when compared with taxane studies [12–16, 18].

A retrospective review of seven phase 2 and phase 3 trastuzumab clinical trials reported that the patients treated with trastuzumab were at an increased risk of cardiac dysfunction. The incidence of cardiac dysfunction was highest in patients receiving concomitant trastuzumab and anthracycline/cyclophosphamide (27 %). The risk was substantially lower

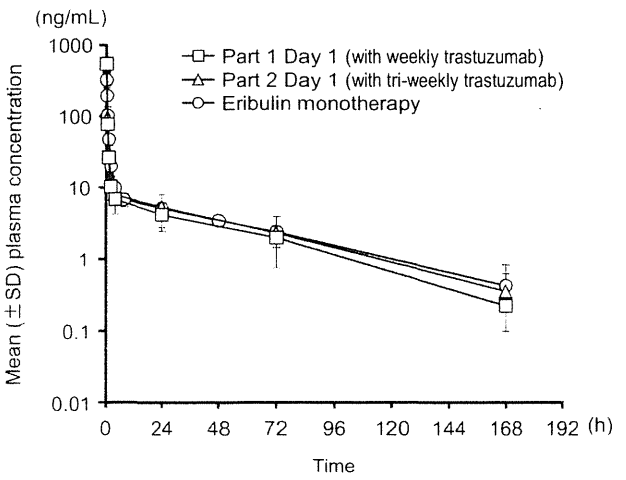

Fig. 2 PK analysis of the relationship between mean plasma eribulin concentration versus time profiles for Parts 1 and 2 of the study and for a previous phase 1 study [12] (cycle 1, day 1)

Table 4 Pharmacokinetic parameters of eribulin (cycle 1, day 1)

PK Parameter (unit)	Part 1 (n=6)	Part 2 (n=6)	Monotherapy (n=6)
C_{max} (ng/mL)	547±128	582±61.0	519.4±107.2
$AUC_{(0\text{-}inf)}$ (ng·h/mL)	524±137	631±271	672.7±113.7
$t_{1/2}$ (h)	38.1±7.80	35.0±10.8	39.4±8.3
CL (L/h/m^2)	2.47±0.774	2.12±0.754	1.89±0.33
V_{ss} (L/m^2)	101±45.3	69.8±11.8	76.3±19.2

Mean±SD

$AUC_{(0\text{-}inf)}$ area under the concentration-time curve from time zero to infinity, CL total clearance, C_{max} maximum plasma concentration, $t_{1/2}$ terminal half-life, V_{ss} steady-state volume of distribution

in patients receiving paclitaxel and trastuzumab (13 %), trastuzumab alone (3 to 7 %), anthracycline/cyclophosphamide alone (8 %), or paclitaxel alone (1 %) [19]. To evaluate the risk of cardiac dysfunction in patients receiving the combination therapy of eribulin mesylate and trastuzumab, periodic assessments of QTcF and LVEF were conducted. Although an ejection fraction decreased (grade 2) was observed in two patients (16.7 %), these patients recovered after 1 week without treatment. The mean LVEF transition was ≥55 % in either part 1 or 2 of the study (Fig. 1). The other cardiac disorders experienced by patients were second-degree atrioventricular block and palpitation, which did not require treatment. Therefore, a clear increased risk of severe cardiac dysfunction resulting from the addition of eribulin mesylate to trastuzumab was not suggested from this study. Since a few cardiac events were observed, cardiac function should be routinely assessed in patients receiving eribulin mesylate in combination with trastuzumab, which is consistent with the recommendation for patients receiving trastuzumab in other combination therapies. The neutropenia observed was manageable, and the non-hematologic AEs were generally mild. Thus, treatment with eribulin mesylate (1.4 mg/m^2) on days 1 and 8 of a 21-day cycle with appropriate dose adjustment was regarded as tolerable in combination with trastuzumab (either weekly or tri-weekly) in Japanese patients.

Table 5 Pharmacokinetic parameters of trastuzumab (cycle 1, day 1)

PK parameter (unit)	Part 1 (4 mg/kg) (n=6)	Part 2 (8 mg/kg) (n=6)
C_{max} (μg/mL)	74.9±25.9	194±33.1
AUC (μg·h/mL)	10900±868	29800±3740
$t_{1/2}$ (h)	115±28.0	173±26.7
CL (mL/h/kg)	0.369±0.0297	0.271±0.0343
V_{ss} (mL/kg)	62.4±17.9	62.0±4.04

Mean±SD

$AUC_{(0\text{-}inf)}$ area under the concentration-time curve from time zero to infinity, CL total clearance, C_{max} maximum plasma concentration, $t_{1/2}$ terminal half-life, V_{ss} steady-state volume of distribution

Table 6 Best tumor responses

	Part 1	Part 2	Total
Response category, n (%)	*N*=6	*N*=6	*N*=12
Best tumor response, n (%)			
CR	0	0	0
PR	1 (16.7)	0	1 (8.3)
SD (including non-CR/non-PD)	4 (66.7)	6 (100.0)	10 (83.3)
PD	1 (16.7)	0	1 (8.3)
Not evaluable	0	0	0
Objective response rate	1 (16.7)	0	1 (8.3)
95 % CI	0.4, 64.1	0.0, 45.9	0.2, 38.5
Disease Control Rate	5 (83.3)	5 (83.3)	10 (83.3)
95 % CI	35.9, 99.6	35.9, 99.6	51.6, 97.9
Clinical Benefit Rate	4 (66.7)	2 (33.3)	6 (50.0)
95 % CI	22.3, 95.7	4.3, 77.7	21.1, 78.9

CI confidence interval, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

The PK profile of eribulin in combination with trastuzumab was similar between the present study and the previously reported phase 1 study of eribulin mesylate monotherapy in Japanese patients [12], including the mean $t_{1/2}$, volume of distribution, and renal and systemic clearance (Fig. 2, Table 4), and as in those studies, the parameters were consistent between days 1 and 8 of the first cycle. The PK profile of trastuzumab (Table 5) was also similar to that in the previous reported phase 1 study of trastuzumab [20]. Combination therapy of trastuzumab with eribulin mesylate did not appear to change the PK profile of either eribulin or trastuzumab. Therefore, no pharmacokinetic drug-drug interaction between eribulin and trastuzumab was indicated.

Although efficacy was not a primary objective, 8 of 12 patients completed more than 5 cycles of treatment and the clinical benefit rate (CBR) was 50.0 % (95 % CI: 21.1, 78.9), which also supports the long-term efficacy and safety of this combination therapy. Recently, the final results of a phase 2 study of eribulin mesylate (1.4 mg/m^2) in combination with trastuzumab (8 mg/kg as a loading dose followed by a 6 mg/kg tri-weekly dose) as a first-line therapy for locally recurrent or metastatic HER2+ breast cancer was reported. Those results showed: ORR of 71.2 % (37/52 patients), CBR of 84.6 % (95 % CI: 71.9, 93.1), median progression free survival of 11.6 months (95 % CI: 9.1, 13.9) and an acceptable safety profile. The most common AEs of grade 3 or 4 were neutropenia (38.5 %) with clinical laboratory hematologic abnormalities of neutrophils (55.8 %) and peripheral neuropathy (26.9 %) [21]. The low incidence of severe neutropenia compared with that of the present study (100 %) was considered to be due to the difference of prior chemotherapy regimen numbers. The high incidence of severe peripheral neuropathy compared with that of the present study (8.3 %) was likely due to the prolonged duration of treatment in a first-line setting.

In conclusion, 1.4 mg/m^2 eribulin mesylate administered on days 1 and 8 of a 21-day cycle in combination with either weekly or tri-weekly trastuzumab was well tolerated in extensively pre-treated Japanese patients with advanced or recurrent HER2+ breast cancer. The safety profile shown in the present study and the reported phase 2 study [21] indicates that further evaluation of eribulin mesylate and trastuzumab combination therapy is warranted.

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Ethical standards The protocol was approved by the Institutional Review Board and conducted in accordance with the Declaration of Helsinki.

Conflict of interest Hirofumi Mukai received lecture fees from Eisai Co., Ltd., AstraZeneca K.K., Chugai Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., Sanofi K.K., and Taiho Pharmaceutical Co., Ltd. Toshiaki Saeki received lecture fees from Eisai Co., Ltd., AstraZeneca K.K., Bayer Yakuhin, Ltd., Chugai Pharmaceutical Co., Ltd., Fuji Pharma Co., Ltd., Kyowa Hakko Kirin Co., Ltd., Mochida Pharmaceutical Co., Ltd., Novartis Pharma K.K., Ono Pharmaceutical Co., Ltd., Pfizer Japan Inc., Sanofi K.K. and Taiho Pharmaceutical Co., Ltd. Yasutsuna Sasaki received lecture fees from Eisai Co., Ltd. and Chugai Pharmaceutical Co., Ltd. Hirofumi Mukai, Yoichi Naito and Nobuaki Matsubara received research funding from Eisai Co., Ltd., Chugai Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., Glaxo SmithKline K.K., Nippon Boehringer Ingelheim Co., Ltd., Nippon Kayaku Co., Ltd., Novartis Pharma K.K., Phizer Japan Inc. and the Public Health Research Foundation. Toshiaki Saeki received research funding from Eisai Co., Ltd., Chugai Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., Kyowa Hakko Kirin Co., Ltd., Novartis Pharma K.K., Ono Pharmaceutical Co., Ltd., Taiho Pharmaceutical Co., Ltd., Takeda Pharmaceutical Company Limited. Yasutsuna Sasaki received research funding from Eisai Co., Ltd. and Chugai Pharmaceutical Co., Ltd. Tadashi Nakanishi and Hiroshi Obaishi are employees and own stock of Eisai Co., Ltd. Masayuki Namiki is an employee of Eisai Co., Ltd. Ken Shimada has no disclosures. The study was designed under the responsibility of Eisai Co., Ltd.; The study was funded by Eisai Co., Ltd.; Eribulin mesylate was provided by Eisai Co.,

Ltd.; Eisai Co., Ltd. collected and analyzed the data and contributed to the interpretation of the study. All authors had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

References

1. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A et al (1989) Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244:707–712
2. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235:177–182
3. Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S, Stewart SJ, Press M (2002) Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 20:719–726
4. Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, Wolter JM, Paton V, Shak S, Lieberman G, Slamon DJ (1999) Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 17:2639–2648
5. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 344:783–792
6. Baselga J, Carbonell X, Castañeda-Soto NJ, Clemens M, Green M, Harvey V, Morales S, Barton C, Ghahramani P (2005) Phase II study of efficacy, safety, and pharmacokinetics of trastuzumab monotherapy administered on a 3-weekly schedule. J Clin Oncol 23:2162–2171
7. Leyland-Jones B, Gelmon K, Ayoub JP, Arnold A, Verma S, Dias R, Ghahramani P (2003) Pharmacokinetics, safety, and efficacy of trastuzumab administered every three weeks in combination with paclitaxel. J Clin Oncol 21:3965–3971
8. Bullock K, Blackwell K (2008) Clinical efficacy of taxane-trastuzumab combination regimens for HER-2-positive metastatic breast cancer. Oncologist 13:515–525. doi:10.1634/theoncologist.2007-0204
9. Redana S, Donadio M, Nolè F, Jacomuzzi ME, Beano A, Martinello R, Sapino A, Viale G, Aglietta M, Montemurro F (2010) Trastuzumab with either docetaxel or vinorelbine as first-line treatment for patients with HER2-positive advanced breast cancer: a retrospective comparison. BMC Cancer 10:28. doi:10.1186/1471-2407-10-28
10. Kuznetsov G, Towle MJ, Cheng H, Kawamura T, TenDyke K, Liu D, Kishi Y, Yu MJ, Littlefield BA (2004) Induction of morphological and biochemical apoptosis following prolonged mitotic blockage by halichondrin B macrocyclic ketone analog E7389. Cancer Res 64:5760–5766
11. Towle MJ, Salvato KA, Budrow J, Wels BF, Kuznetsov G, Aalfs KK, Welsh S, Zheng W, Seletsky BM, Palme MH, Habgood GJ, Singer LA, Dipietro LV, Wang Y, Chen JJ, Quincy DA, Davis A, Yoshimatsu K, Kishi Y, Yu MJ, Littlefield BA (2001) In vitro and in vivo anticancer activities of synthetic macrocyclic ketone analogues of halichondrin B. Cancer Res 61:1013–1021
12. Mukohara T, Nagai S, Mukai H, Namiki M, Minami H (2012) Eribulin mesylate in patients with refractory cancers: a Phase I study. Investig New Drugs 30:1926–1933. doi:10.1007/s10637-011-9741-2
13. Aogi K, Iwata H, Masuda N, Mukai H, Yoshida M, Rai Y, Taguchi K, Sasaki Y, Takashima S (2012) A phase II study of eribulin in Japanese patients with heavily pretreated metastatic breast cancer. Ann Oncol 23:1441–1448. doi:10.1093/annonc/mdr444
14. Vahdat LT, Pruitt B, Fabian CJ, Rivera RR, Smith DA, Tan-Chiu E, Wright J, Tan AR, Dacosta NA, Chuang E, Smith J, O'Shaughnessy J, Shuster DE, Meneses NL, Chandrawansa K, Fang F, Cole PE, Ashworth S, Blum JL (2009) Phase II study of eribulin mesylate, a halichondrin B analog, in patients with metastatic breast cancer previously treated with an anthracycline and a taxane. J Clin Oncol 27:2954–2961. doi:10.1200/JCO.2008.17.7618
15. Cortes J, Vahdat L, Blum JL, Twelves C, Campone M, Roché H, Bachelot T, Awada A, Paridaens R, Goncalves A, Shuster DE, Wanders J, Fang F, Gurnani R, Richmond E, Cole PE, Ashworth S, Allison MA (2010) Phase II study of the halichondrin B analog eribulin mesylate in patients with locally advanced or metastatic breast cancer previously treated with an anthracycline, a taxane, and capecitabine. J Clin Oncol 28:3922–3928. doi:10.1200/JCO.2009.25.8467
16. Cortes J, O'Shaughnessy J, Loesch D, Blum JL, Vahdat LT, Petrakova K, Chollet P, Manikas A, Diéras V, Delozier T, Vladimirov V, Cardoso F, Koh H, Bougnoux P, Dutcus CE, Seegobin S, Mir D, Meneses N, Wanders J, Twelves C (2011) Eribulin monotherapy versus treatment of physician's choice in patients with metastatic breast cancer (EMBRACE): a phase 3 open-label randomised study. Lancet 377:914–923. doi:10.1016/S0140-6736(11)60070-6
17. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45:228–247. doi:10.1016/j.ejca.2008.10.026
18. Markman M (2003) Management of toxicities associated with the administration of taxanes. Expert Opin Drug Saf 2:141–146
19. Seidman A, Hudis C, Pierri MK, Shak S, Paton V, Ashby M, Murphy M, Stewart SJ, Keefe D (2002) Cardiac dysfunction in the trastuzumab clinical trials experience. J Clin Oncol 20:1215–1221
20. Tokuda Y, Watanabe T, Omuro Y, Ando M, Katsumata N, Okumura A, Ohta M, Fujii H, Sasaki Y, Niwa T, Tajima T (1999) Dose escalation and pharmacokinetic study of a humanized anti-HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. Br J Cancer 81:1419–1425
21. Wilks S, Puhalla S, O'Shaughnessy J, Schwartzberg L, Berrak E, Song J, Cox D, Vahdat L (2014) Phase 2, multicenter, single-arm study of eribulin mesylate with trastuzumab as first-line therapy for locally recurrent or metastatic HER2-positive breast cancer. Clin Breast Cancer. doi:10.1016/j.clbc.2014.04.004

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Original article

Pathologic complete response after neoadjuvant chemotherapy in HER2-overexpressing breast cancer according to hormonal receptor status

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ABSTRACT

Objective: For patients with HER2-positive breast cancer, the prognostic impact of pathologic complete response (pCR) after neoadjuvant chemotherapy (NAC) is unclear when stratified by hormonal receptor (HR) status; however, the impact of pCR on survival when stratified by hormonal receptor (HR) status is uncertain.

Patients and methods: This multicenter retrospective study investigated the predictors of pCR and its prognostic value in Japanese patients 366 HER2-positive breast cancer who received NAC. pCR was defined as no invasive residual tumor in the breast or axilla.

Results: Median follow-up was 55 months. Multivariate analysis revealed that HR status (OR, 0.37; $p < 0.001$) was one of the independent predictors of pCR. Five-year recurrence-free survival was higher in HR-negative patients with pCR (93%) than in those without pCR (68%), and pCR was independently prognostic (hazard ratio, 0.32; $p = 0.005$). However, 5-year recurrence-free survival was not different between HR-positive patients with pCR (94%) and those without pCR (84%), and pCR was not significantly prognostic (hazard ratio, 0.53; $p = 0.39$). In addition, 5-year overall survivals were high and similar (97% in pCR, 94% in non-pCR). Among 204 patients treated with neoadjuvant trastuzumab, pCR was not significantly prognostic in the HR-positive group (hazard ratio, 0.63; $p = 0.56$).

Conclusion: Our study suggested that the HER2-positive HR-positive patients had a good prognosis despite the lower achievement rate of pCR, whose prognostic impact was smaller than that in the HER2-positive HR-negative patients. The treatment strategy for HER2-positive breast cancer can be stratified by HR status.

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Introduction

Neoadjuvant chemotherapy (NAC) has been shown to be as effective as adjuvant chemotherapy [1] and now represents an option for patients with operable breast cancer in order to downstage surgical requirement, to evaluate chemosensitivity and to facilitate translational research [2]. Pathologic complete response (pCR) after NAC predicted long-term outcome in several neoadjuvant studies [3–6] and is therefore a potentially valuable surrogate endpoint for survival. The Food and Drug Administration (FDA) may grant accelerated approval of new drugs for neoadjuvant breast-cancer treatment on the basis of the rate of pCR as a surrogate endpoint that is reasonably likely to predict clinical benefit [7].

However, pCR rates in NAC studies have varied widely and some studies failed to show an association between pCR rate and improved outcome [8]. One of the reasons for these discrepancies is the variation of pCR in the incidence and its association with survival among various breast cancer–intrinsic subtypes [4]. Patients with luminal A–like hormonal receptor (HR)-positive breast cancer show a low pCR rate, and their overall prognosis is favorable, whereas patients with triple-negative breast cancer show a high pCR rate but have an unfavorable outcome [9]. The type of NAC agent also might affect pCR rate. In human epidermal growth factor receptor 2 (HER2)-overexpressing patients, the addition of trastuzumab to chemotherapy demonstrated a more than doubled pCR rate compared to chemotherapy alone [10]. In addition, the different definitions of pCR among trial groups have also caused different pCR rates. Some trials have applied the definition of pCR to the breast tumor only [5,6], while others have included the axillary nodes [11,12]. Furthermore, some studies have included the presence of noninvasive cancer residuals in their definition of pCR [11].

As for HER2-positive breast cancer, a recent meta-analysis [13] reported that the pCR rate among HER2-positive HR-positive tumors was higher than that among HER2-negative HR-positive tumors and lower than that among HER2-positive HR-negative tumors. However, this result was not adjusted with other important variables such as age and stage. Moreover, although some studies demonstrated a correlation between achievement of pCR and recurrence-free survival (RFS) [14,15], another meta-analysis [13] showed that when stratified by HR status, pCR among HER2-positive HR-positive breast cancer patients did not predict survival although pCR in HER2-positive HR-negative patients significantly correlated with survival. Therefore, in order to clarify the predictive value of HR on pCR and the prognostic impact of pCR on survival in patients with HER2-positive HR-positive breast cancer subtype, we investigated Japanese HER2-positive patients who received neoadjuvant chemotherapy.

Patients and methods

This retrospective study was conducted in accordance with the Declaration of Helsinki. This study was approved by the local institutional review board of each participating institution.

Patients

This multicenter retrospective study included 366 evaluable patients with HER2-positive stage I–III breast cancer receiving neoadjuvant chemotherapy at six institutions between 2003 and 2010. The numbers of eligible patients at each institution were 163 at Hyogo Cancer Center, 77 at National Cancer Center Hospital East, 53 at Toranomon Hospital, 37 at Kinki University School of Medicine, 18 at Hiroshima Prefectural Hospital, and 18 at Saitama Medical University. The follow-up period was completed in October 2012. Individual patient data regarding baseline characteristics, histopathologic results at surgery, and follow-up were extracted for this analysis from the medical charts at each institution.

All eligible patients were confirmed to have *HER2*-overexpressing invasive carcinoma histologically by core needle biopsy. The histologic type of the primary tumor was classified according to the General Rules for Clinical and Pathological Recording of Breast Cancer, The Japanese Breast Cancer Society (2004). *HER2*-overexpression status was defined as a 3 + staining intensity, that is, more than 30% of cancer cells were markedly positive by immunohistochemistry (IHC) using the DAKO HerceptTest (DAKO, Glostrup, Denmark), HER2 kit (Nichirei, Tokyo, Japan), CB11 (Novocastra, Newcastle Upon Tyne, UK) or 4B5 (Roche, Basel, Switzerland), or a 2 + staining intensity for *HER2* gene amplification, that is, a HER2/CEP17 signal ratio of 2.0 by fluorescent in situ hybridization (FISH; PathVision Abbott, Chicago, IL). The levels of estrogen receptor (ER; 6F11, Novocastra, Newcastle Upon Tyne, UK, or SP1, Roche, Basel, Switzerland) and progesterone receptor (PgR; 1A6, Novocastra, Newcastle Upon Tyne, UK, or 1E2, Roche, Basel, Switzerland) were measured by IHC analysis of paraffin-embedded tissue specimens. ER and PgR were classified as positive if more than 1% of cancer cell nuclei were stained by the respective antibody, regardless of the staining intensity. All IHC and FISH analyses were performed at each institution. Primary tumors were either ≥1 cm based on clinical or ultrasound assessment or were diagnosed clinically as inflammatory breast cancer or occult breast cancer. Patients did not have distant metastases, as confirmed by chest x-ray, sonography or computed tomography of the upper abdomen, and a bone scan. Eastern Cooperative Oncology Group (ECOG) performance status had to be lower than 2.

Small central review of immunohistochemical ER and HER2 stains

Several antibodies were used for immunohistochemical analysis of ER and HER2. Therefore, we conducted a mini-review using 82 ER and 61 HER2 immunohistochemistry stains from 84 patients of five institutions and compared clinically significant discordance. All the specimens were reviewed by a single pathologist (Dr. Sakuma) at Hyogo Cancer Center. The 84 patients comprised 23 at Toranomon Hospital, 21 at Kinki University School of Medicine, 18 at Hiroshima Prefectural Hospital, 13 at Hyogo Cancer Center and 9 at Saitama Medical University.

Treatment

All of the patients received neoadjuvant anthracycline and/or taxane. Trastuzumab was administered concomitantly with taxanes. Trastuzumab was also administered post-operatively after approval for its use in an adjuvant setting in Japan in 2007. Treatment after surgery, including chemotherapy and hormonal therapy, was at the physician's discretion. Patients with ER-and/or PgR-positive tumors should receive adjuvant endocrine treatment for at least 5 years. Adjuvant radiotherapy was recommended for patients who underwent breast-conserving surgery as well as for patients who underwent mastectomy but had initial stage cT4 or cN3 disease.

Endpoints

A wide range of criteria have been used to define pCR and a consensus has yet to be reached. In this study, pCR at surgery was

Table 1
Patient characteristics.

Characteristics	All $n = 366$ (%)	HR-negative $n = 190$ (%)	HR-positive $n = 176$ (%)	Trastuzumab $n = 204$ (%)
Median follow-up, months	55 (9–104)	52 (9–113)	60 (10–115)	45 (10–115)
Median age, years	54 (25–84)	56 (31–84)	50 (25–75)	55 (28–84)
<50 years	130 (36)	46 (24)	84 (48)	74 (36)
Histology				
Invasive ductal carcinoma	352 (96)	184 (97)	168 (95)	196 (96)
Invasive lobular carcinoma	4 (1)	2 (1)	2 (1)	2 (1)
Other	10 (3)	4 (2)	6 (3)	6 (3)
Primary tumor				
T1–2	90 (25)	54 (28)	36 (20)	68 (33)
T3–4	276 (75)	136 (72)	140 (80)	136 (67)
Axillary node				
N0	128 (35)	55 (15)	73 (41)	55 (27)
N1–3	238 (65)	135 (85)	103 (59)	149 (73)
Stage				
I	16 (4)	10 (5)	6 (3)	12 (6)
II	226 (62)	105 (55)	121 (69)	100 (49)
III	124 (34)	75 (39)	49 (28)	92 (45)
Hormonal receptor status				
ER positive	169 (46)	0	169 (96)	84 (41)
PgR positive	123 (34)	0	123 (70)	69 (34)
ER or PgR positive	176 (48)	0	176 (100)	91 (45)
Neoadjuvant regimens				
Anthracycline	271 (74)	147 (77)	124 (72)	145 (71)
Taxane	327 (89)	178 (94)	149 (85)	204 (100)
Trastuzumab	204 (56)	113 (59)	91 (52)	204 (100)
pCR				
ypT0 ypN0	88 (24)	65 (34)	23 (13)	60 (29)
ypT0/is ypN0	130 (36)	93 (49)	37 (21)	88 (43)
ypT0/is ypN0/+	137 (37)	97 (51)	40 (23)	92 (45)
Trastuzumab (1 yr)[b]	219 (60)	121 (64)	98 (56)	162 (79)
Adjuvant hormonal therapy	173 (47)	9[a](5)	164 (45)	86 (42)
Adjuvant chemotherapy	82 (22)	29 (12)	53 (30)	20 (11)

Abbreviations: HR, hormonal receptor; ER, estrogen receptor; PgR, progesterone receptor.
[a] Nine patients with HER2+HR-tumor received adjuvant hormonal therapy according to the HR of the operative tissue.
[b] Patients who received trastuzumab before and after surgery for a total of one year.

defined as no evidence of invasive or noninvasive residual cells in the breast or axillary nodes (ypT0 ypN0), no evidence of invasive residual cells in the breast or axillary nodes; noninvasive breast residuals allowed (ypT0/is ypN0), and no evidence of invasive residual cells in the breast; noninvasive breast residuals and infiltrated lymph nodes allowed (ypT0/is ypN0/+). In the main analyses of this study, pCR was defined as ypT0/is ypN0 in line with the MD Anderson Cancer Center criteria [11] which were recommended by the Breast International Group and the National Cancer Institute-sponsored North American Breast Cancer Group [16]. Because the presence of residual ductal carcinoma in situ (DCIS) after preoperative therapy does not influence the long-term rate of local recurrence or overall survival [17], we included patients with residual DCIS in the category of pCR. Additional analyses using different pCR definitions were performed for better comparison with the literature.

RFS was measured from the date of initial diagnosis to the date of recurrence (including locoregional recurrence) or the last follow-up visit. Overall survival (OS) was measured from the starting date of neoadjuvant chemotherapy to the date of last contact or death from any cause. For the survival analysis, data on surviving patients were censored on the date of their last follow-up examination. The locoregional or distant recurrences were evaluated on physical examination or by radiological imaging.

Statistical analysis

Analysis of the predictive factors for pCR among the baseline parameters was performed by logistic regression analysis. Variables with p-value ≤ 0.10 on univariate analysis and a clinically important variable (age) were included in the multivariate analysis.

RFS and OS according to pCR status were plotted as Kaplan–Meier curves. The log-rank test was used to identify predictive factors associated with pCR. Multivariate analysis with a Cox proportional-hazards model including all clinically important parameters was used to identify independent prognostic factors

Table 2
Logistic regression analysis of predictors of pCR (ypT0/is ypN0).

		$n = 366$	Univariate	Multivariate	Odds ratio (95% CI)
			p value	p value	
Age (yr)	≥50	241	0.55	0.063	0.70
	<50	125			(0.42–1.17)
Primary tumor	T1–2	90	0.81		
	T3–4	276			
Nodal status	N0	126	0.73		
	N1–3	240			
Stage	I–II	242	0.35		
	III	124			
Hormonal	Negative	190	<0.001	<0.001	0.34
receptor status	Positive	176			(0.21–0.55)
Anthracycline	No	92	0.008	0.006	2.22
	Yes	274			(1.26–3.90)
Taxane	No	42	0.001	0.011	5.15
	Yes	324			(1.46–18.2)
Trastuzumab	No	162	0.001	0.027	1.76
	Yes	204			(1.07–2.90)

Variables tested for inclusion in the multivariate logistic regression model were age <50 years, hormonal receptor status, anthracycline, taxane, and trastuzumab. CI, confidence interval.

according to HR status. p-values <0.05 were considered statistically significant.

Among the 204 patients receiving neoadjuvant trastuzumab, the same analyses on predictive factors of pCR and prognostic impact of pCR on survival were performed. All statistical analyses were performed using IBM SPSS Statistics 20 (Armonk, NY, USA).

Results

Baseline patient characteristics

Table 1 shows the patients and tumor characteristics. In the current pooled analysis, the 366 patients with HER2-overexpressing tumor were composed of 176 HR-positive and 190 HR-negative patients. During a median follow-up of 55 months (range, 9–115 months), 65 relapses (18%) and 32 deaths (9%) were observed. Tumors stained positive for ER in 169 (46%) and for PgR in 123 patients (34%), then 176 (48%) were regarded as HR-positive. Ninety-three percent of the patients with HR-positive tumor received adjuvant hormonal therapies. A total of 219 (60%) patients received trastuzumab for one year during neoadjuvant and adjuvant therapies. The number of the patients who achieved pCR as defined by ypT0 ypN0, ypT0/is ypN0, or ypT0/is ypN0/+ were 88 (24%), 130 (36%), or 137 (37%), respectively. The small central review of immunohistochemical ER and HER2 stains showed 6.1% (5/82) and 1.6% (1/61) discordance when examining ER and HER2 slides, respectively. In one case, ER-negative PgR-positive status changed to ER-positive, and in one case, HER2 2+ (FISH-positive) status changed to HER2 3+. Excluding these two cases, clinically significant discordance rates were 4.9% (4/81) in ER stains and 0% (0/60) in HER2 stains.

Predictors of pCR

The pCR rate in patients with HR-positive tumors was significantly lower than that in patients with HR-negative tumors [21% (37/176) vs. 49% (93/190); $p < 0.001$]. HR status [OR, 0.32 (0.19–0.54); $p < 0.001$] was one of the independent predictors for pCR (ypT0/is ypN0) in multivariate analysis (Table 2). HR status was also the independent predictor by the same analysis using other definitions of pCR [ypT0 ypN0; OR, 0.37 (0.21–0.64), $p < 0.001$ and ypT0/is ypN0/+; OR, 0.35 (0.22–0.56), $p < 0.001$].

Correlation between pCR and outcome

Among patients with HR-negative tumors, the hazard ratio for RFS comparing patients with or without pCR defined as ypT0isN0 was 0.30 (95% CI, 0.14 to 0.63; $p = 0.002$), and the five-year RFS was significantly higher in patients with pCR (93%) than in patients with non-pCR (68%) (Fig. 1A). The five-year OS were also significantly different between patients with pCR and non-pCR (Fig. 1C). On the other hand, among patients with HER2-positive HR-positive tumor, the hazard ratio for RFS comparing patients with or without pCR was 0.57 (95% CI, 0.17 to 1.90; $p = 0.36$), the five-year RFS rates were not significantly different between patients with pCR (94%) and those without pCR (84%) (Fig. 1B) and the five-year OS rates were high and similar (97% in patients with pCR, 94% in patients without pCR; Fig. 1D).

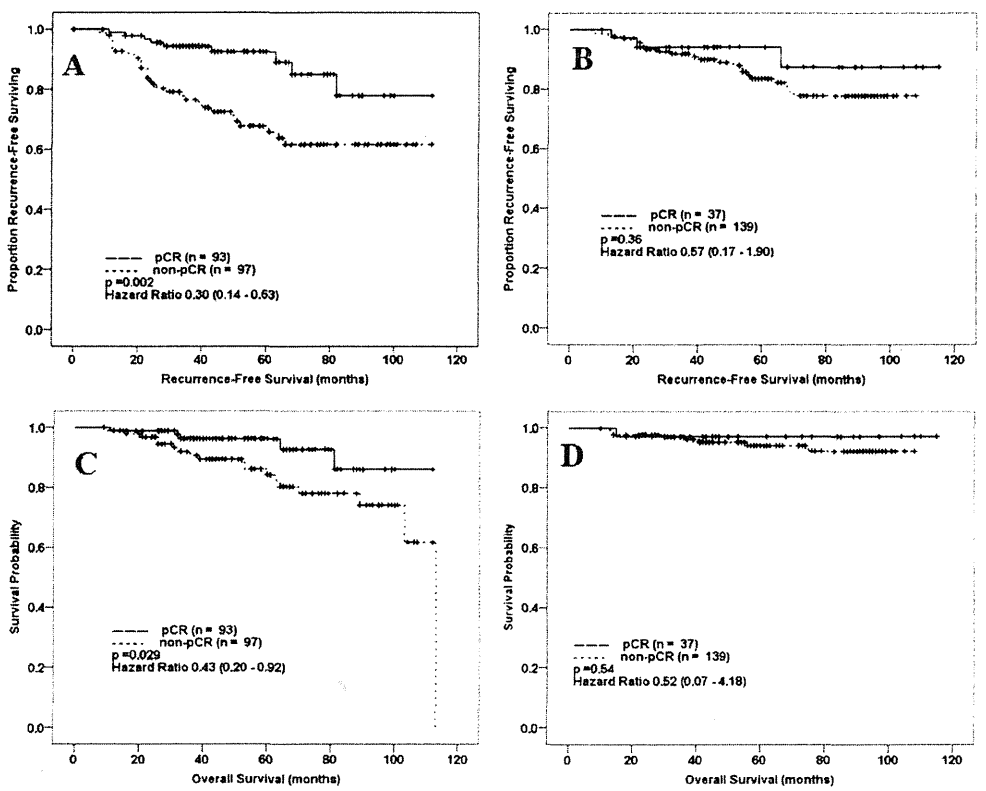

Fig. 1. Prognostic impact of pathologic complete response (pCR) on survival in 366 patients according to hormonal receptor (HR) status. A: Recurrence-free survival in patients with HER2-positive HR-negative tumor. B: Recurrence-free survival in patients with HER2-positive HR-positive tumor. C: Overall survival in patients with HER2-positive HR-negative tumor. D: Overall survival in patients with HER2-positive HR-positive tumor.

Table 3
Multivariate analysis of prognostic factors on RFS according to HR status.

		HER2-positive/HR-negative			HER2-positive/HR-positive		
		$n = 190$	p value	Harzard ratio (95% CI)	$n = 176$	p value	Harzard ratio (95% CI)
Age (yr)	≥50	154	0.84	1.09 (0.49–2.42)	92	0.72	1.15 (0.52–2.54)
	<50	36			84		
Stage	I–II	115	0.02	2.24 (1.13–4.44)	127	0.008	3.10 (1.34–7.18)
	III	75			49		
Anthracycline	No	43	0.62	0.84 (0.41–1.72)	52	0.95	0.74 (0.30–3.06)
	Yes	147			124		
Taxane	No	12	0.19	0.46 (0.14–1.48)	25	0.39	2.21 (0.36–13.8)
	Yes	178			149		
Trastuzumab (1 yr)[a]	No	69	0.91	0.96 (0.48–1.93)	78	0.46	1.47 (0.53–4.11)
	Yes	121			98		
Adjuvant HT	No	181	0.10	2.53 (0.85–7.51)	12	<0.001	0.12 (0.05–0.30)
	Yes	9			164		
Adjuvant chemotherapy	No	161	0.51	0.72 (0.27–1.94)	123	0.472	1.29 (0.33–5.09)
	Yes	29			53		
pCR	No	97	0.005	0.32 (0.15–0.71)	139	0.39	0.53 (0.15–1.96)
	Yes	93			37		

Abbreviations: HR, hazard ratio; CI, confidence interval; HT, hormonal therapy; pCR, pathologic complete response (ypT0/is ypN0).
[a] Patients who received trastuzumab before and after surgery for a total of one year.

Multivariate analysis of prognostic factors on DFS

In HER2-positive HR-negative patients, the independent prognostic factors by multivariate analysis were pCR (Hazard ratio, 0.32; $p = 0.005$) and stage III (Hazard ratio, 2.24; $p = 0.023$). However, when confined to patients with HER2-positive HR-positive tumors, multivariate analysis demonstrated that stage III and adjuvant hormonal therapy were independently correlated with RFS and pCR (HR, 0.53; $p = 0.39$) was not (Table 3).

Among HER2-positive HR-positive patients, 11% (4/37) of pCR patients received adjuvant cytotoxic chemotherapy, while 35% (49/139) of non-pCR patients received it. This result might influence the prognostic impact of pCR; therefore, excluding 82 patients (53 with HR-positive tumor, 29 with HR-negative tumor) who received adjuvant cytotoxic chemotherapy, we analyzed the data of 284 patients. The only independent prognostic factor was pCR (Hazard ratio, 0.39; 95% CI, 0.17 to 0.88; $p = 0.023$) in HER2-positive HR-negative patients. However, in HER2-positive HR-positive patients, only adjuvant hormonal therapy was independently correlated with RFS, and pCR (HR, 0.63; 95% CI, 0.15 to 2.70; $p = 0.53$) was not.

Analyses among patients who received neoadjuvant trastuzumab

Among 204 patients treated with neoadjuvant trastuzumab, all received taxane concomitantly. One hundred sixty-two (79%) received trastuzumab for a total of one year. During a median follow-up of 45 months (range, 10–115 months), 36 relapses (18%) and 15 deaths (7%) were observed. HR status (OR, 0.36; 95% CI, 0.18 to 0.71; $p < 0.001$) was one of the independent predictors of pCR as defined by ypT0/is ypN0. Among patients with HER2-positive HR-negative tumors, the hazard ratio for RFS comparing patients with or without pCR as defined by ypT0/is ypN0 was 0.31 (95%CI, 0.14 to 0.66; $p = 0.003$) (Table 4), and the five-year RFS was significantly higher in patients with pCR than in patients without pCR (91% vs. 66%) (Fig. 2A). On the other hand, among patients with HER2-positive HR-positive tumors, again, the hazard ratio for RFS comparing patients with or without pCR as defined by ypT0/is ypN0 was 0.63 (95%CI, 0.14 to 2.92; $p = 0.56$) (Table 4), and 5-year RFS did not significantly differ between patients with pCR (90%) and those without pCR (73%) (Fig. 2B).

Table 4
Multivariate analysis of prognostic factors for RFS according to HR status among patients who received neoadjuvant trastuzumab.

		HER2-positive/HR-negative			HER2-positive/HR-positive		
		$n = 113$	p value	Harzard ratio (95% CI)	$n = 91$	p value	Harzard ratio (95% CI)
Age (yr)	≥50	85	0.91	0.96 (0.44–2.10)	45	0.80	1.14 (0.40–3.25)
	<50	28			46		
Stage	I–II	59	0.033	2.05 (1.06–3.97)	53	0.013	4.07 (1.34–12.4)
	III	54			38		
Anthracycline	No	31	0.81	0.92 (0.45–1.88)	28	0.67	0.75 (0.20–2.79)
	Yes	82			63		
Taxane	No	0	–	–	0	–	–
	Yes	113			91		
Trastuzumab (1 yr)[a]	No	21	0.96	0.98 (0.49–1.99)	21	0.25	0.49 (0.15–1.65)
	Yes	92			70		
Adjuvant HT	No	107	0.13	2.32 (0.79–6.83)	11	<0.001	0.10 (0.03–0.32)
	Yes	6			80		
Adjuvant chemotherapy	No	104	0.88	0.93 (0.38–2.32)	80	0.62	0.64 (0.11–3.71)
	Yes	9			11		
pCR	No	45	0.003	0.31 (0.14–0.66)	69	0.56	0.63 (0.14–2.92)
	Yes	66			22		

Abbreviations: HR, hormonal receptor; CI, confidence interval; HT, hormonal therapy; pCR, pathologic complete response (ypT0/is ypN0).
[a] Patients who received trastuzumab before and after surgery for a total of one year.

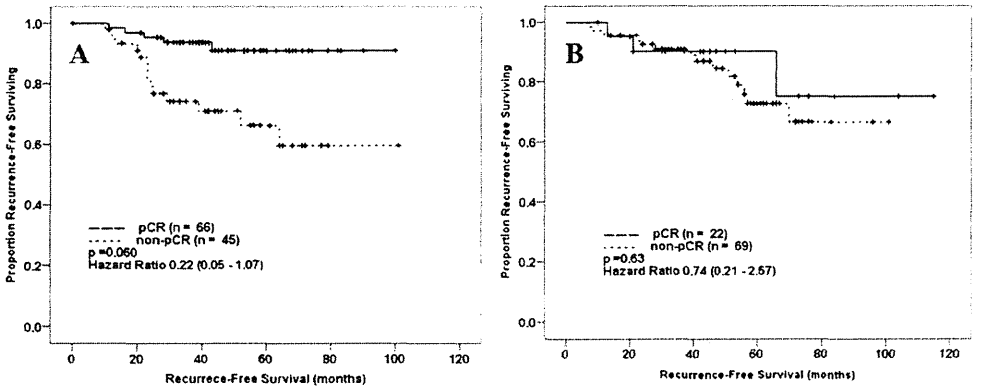

Fig. 2. Prognostic impact of pathologic complete response (pCR) on recurrence-free survival in 204 patients who received neoadjuvant trastuzumab according to hormonal receptor (HR) status. A: Patients with HER2-positive HR-negative tumor. B: Patients with HER2-positive HR-positive tumor.

Discussion

Our study suggested that the prognostic impact of pCR after NAC in patients with HER2-positive HR-positive breast cancer was smaller than that in HER2-positive HR-negative cancer. Although the hazard ratio of 0.53 (Table 3) for RFS among HR-positive subgroup may be apparently meaningful, the absolute differences in 5-year RFS and OS rates between the HR-positive and HR-negative subgroups were only 10% and 3%, respectively. Therefore, the observed lower pCR rate might not predict an unfavorable prognosis of HR-positive subgroup. At the 35th Annual San Antonio Breast Cancer Symposium (SABCS, 2012), Cortazar, et al. presented results of a meta-analysis results of 12,993 breast cancer patients [18]. Among 1989 HER2-positive patients in this meta-analysis, the hazard ratios for event-free survival comparing patients with or without pCR as defined by ypT0isN0 were 0.25 ($p < 0.001$) and 0.58 ($p = 0.001$) in HR-negative and HR-positive subgroups, respectively, which were similar to our results [0.30 in HR-negative (Fig. 1A) and 0.57 in HR-positive (Fig. 1B)]. The hazard ratio in HR-positive patients was again much higher than that in HR-negative patients, and the impact of pCR on overall survival had not been shown in HER2-positive subtype. Therefore, the prognostic impact of pCR in HER2-positive HR-positive patients has not been confirmed yet. Moreover, this meta-analysis failed to demonstrate the magnitude of improvement in pCR rate, which is needed to predict the improvement of recurrence-free or overall survival and the superiority of one regimen over another. Minckwitz [19] stated that confirmation of a quantitative correlation between increments in pCR and gains in survival on large data sets is needed to use the neoadjuvant model for accelerated drug approval. It might be difficult to achieve accelerated drug approval using neoadjuvant models especially in HER2-positive HR-positive breast cancer patients.

In addition, our study also demonstrated that the HR-positive subgroup had a much lower achievement rate of pCR (21%) than HR-negative subgroup (49%). Because of the low achievement rate of pCR and its small prognostic impact, the treatment strategies for HER2-positive HR-positive breast cancer might be reconsidered. By using anti-HER2 and hormonal agents, cytotoxic drugs with strong adverse effects can be omitted without damaging overall survival. Indeed, the TBCRC06 trial demonstrated that neoadjuvant therapy with trastuzumab, lapatinib, and letrozole for HER2-positive HR-positive breast cancer yielded a pCR rate of 21% [20], which was comparable with that of 24% among 91 HER2-positive HR-positive patients who received neoadjuvant trastuzumab in our study. At the 35th SABCS, Giuliano, et al. presented that Bcl-2 increased after neoadjuvant lapatinib in HER2-positive HR-positive patients, but not in HER2-positive HR-negative patients. In addition, inhibition of Bcl-2 by ABT-737 reduced the lapatinib-resistant cell growth [21]. The identification of surrogate endpoint biomarkers such as Bcl-2 for HER2-positive HR-positive tumors is warranted to stratify treatments for HER2-positive patients with HR status.

This study was a retrospective analysis without a central assessment of surgical specimens or HER2 status and lacked a sufficient number of patients. However, our small central review using 82 ER specimens and 61 HER2 specimens from 84 patients showed that clinically significant discordance rates were 4.9% and 0% in ER and HER2 stains, respectively. Therefore, we still believe our results are robust. The follow-up period of 4.6 years was relatively short although events in about four-fifths of the patients have occurred within five years in the HERA tiral [22]. Only 56% of the patients were treated with neoadjuvant trastuzumab, however, the analyses among these 204 patients yielded the same results as those among all 366 patients. Although these limitations do not allow us to make firm recommendations for the clinical significance of pCR in HER2-positive HR-positive breast cancer, some tentative conclusions can be drawn. In patients with HER2-positive breast cancer, the HR-positive subgroup had a good prognosis despite the lower achievement rate of pCR, whose prognostic impact was smaller than that in the HR-negative group. The treatment strategy for HER2-positive breast cancer can be stratified by HR status.

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None.

Conflict of interest statement

Dr. Hironobu Minami has received unrestricted research grant and honoraria for activities as a speaker and a member of committee for clinical studies from Chugai Pharmaceutical, the maker of trastuzumab. The other authors have declared no conflicts of interest.

References

[1] 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(8):2672–85.
[2] Mieog JS, van der Hage JA, van de Velde CJ. Preoperative chemotherapy for women with operable breast cancer. Cochrane Database Syst Rev; 2007. CD005002.

[3] 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(2):460–9.
[4] von Minckwitz G, Untch M, Blohmer JU, Costa SD, Eidtmann H, Fasching PA, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 2012;30(15):1796–804.
[5] Bear HD, Anderson S, Smith RE, Geyer Jr CE, 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(13):2019–27.
[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; 2001:96–102.
[7] Prowell TM, Pazdur R. Pathological complete response and accelerated drug approval in early breast cancer. N Engl J Med 2012;366(26):2438–41.
[8] Rastogi P, Anderson SJ, Bear HD, Geyer CE, Kahlenberg MS, Robidoux A, et al. Preoperative chemotherapy: updates of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27. J Clin Oncol 2008;26(5):778–85.
[9] 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(8):1275–81.
[10] Buzdar AU, Ibrahim NK, Francis D, Booser DJ, Thomas ES, Theriault RL, et al. Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. J Clin Oncol 2005;23(16):3676–85.
[11] Green MC, Buzdar AU, Smith T, Ibrahim NK, Valero V, Rosales MF, et al. Weekly paclitaxel improves pathologic complete remission in operable breast cancer when compared with paclitaxel once every 3 weeks. J Clin Oncol 2005;23(25):5983–92.
[12] von Minckwitz G, Rezai M, Loibl S, Fasching PA, Huober J, Tesch H, et al. Capecitabine in addition to anthracycline- and taxane-based neoadjuvant treatment in patients with primary breast cancer: phase III GeparQuattro study. J Clin Oncol 2010;28(12):2015–23.
[13] Houssami N, Macaskill P, von Minckwitz G, Marinovich ML, Mamounas E. Meta-analysis of the association of breast cancer subtype and pathologic complete response to neoadjuvant chemotherapy. Eur J Cancer 2012;48(18):3342–54.
[14] Untch M, Fasching PA, Konecny GE, Hasmuller S, Lebeau A, Kreienberg R, et al. Pathologic complete response after neoadjuvant chemotherapy plus trastuzumab predicts favorable survival in human epidermal growth factor receptor 2-overexpressing breast cancer: results from the TECHNO trial of the AGO and GBG study groups. J Clin Oncol 2011;29(25):3351–7.
[15] Esserman LJ, Berry DA, DeMichele A, Carey L, Davis SE, Buxton M, et al. Pathologic complete response predicts recurrence-free survival more effectively by cancer subset: results from the I-SPY 1 TRIAL–CALGB 150007/150012, ACRIN 6657. J Clin Oncol 2012;30(26):3242–9.
[16] **Fumagalli D, Bedard PL, Nahleh Z, Michiels S, Sotiriou C, Loi S, et al. A common language in neoadjuvant breast cancer clinical trials: proposals for standard definitions and endpoints. Lancet Oncol 13(6):e240–e248.Pubmed Partial ArticleTitle**
[17] Mazouni C, Peintinger F, Wan-Kau S, Andre F, Gonzalez-Angulo AM, Symmans WF, et al. Residual ductal carcinoma in situ in patients with complete eradication of invasive breast cancer after neoadjuvant chemotherapy does not adversely affect patient outcome. J Clin Oncol 2007;25(19):2650–5.
[18] Cortazar PZL, Untch M, et al. Meta-analysis results from the Collaborative Trials in Neoadjuvant Breast Cancer (CTNeoBC). In: The 35rd annual San Antonio breast cancer symposium, Edition San Antonio, TX; 2012.
[19] von Minckwitz G. Pathologic complete response after neoadjuvant therapy of breast cancer: pitfalls and shortcomings. Breast Cancer Res Treat 2012;132(3):779–80.
[20] Chang JCN, Mayer IA, Forero-Torres A, et al. TBCRC 006: a multicenter phase II study of neoadjuvant lapatinib and trastuzumab in patients with HER2-overexpressing breast cancer. J Clin Oncol 2011;(Suppl):29. Abstr 505.
[21] Giuliano MWY, Gutierrez C, et al. Parallel upregulation of Bcl2 and estrogen receptor (ER) expression in HER2+ breast cancer patients treated with neoadjuvant lapatinib. In: The 35rd annual San Antonio breast cancer symposium, Edition San Antonio, TX; 2012.
[22] Goldhirsch AP-GM, Procter M, et al. HERA TRIAL: 2 years versus 1 year of trastuzumab after adjuvant chemotherapy in women with HER2-positive early breast cancer at 8 years of median follow up. In: The 35rd annual San Antonio breast cancer symposium, Edition San Antonio, TX; 2012.

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CLINICAL TRIAL

Survival outcome and reduction rate of Ki-67 between pre- and post-neoadjuvant chemotherapy in breast cancer patients with non-pCR

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Abstract The research question of this investigation is whether the reduction rate of Ki-67 after neoadjuvant chemotherapy (NAC) could indicate a survival in patients with non-pCR. A total of 455 patients had received NAC, and subsequent surgery was analyzed retrospectively. Patients with non-pCR were divided into three subgroups according to Ki-67 change: High-reduction (the absolute value of Ki-67 was reduced by >80 % compared with that prior to NAC), Low-reduction (the absolute value of Ki-67 was reduced by 0–80 % compared with that prior to NAC), and Increase group (the absolute value of Ki-67 was increased compared with that prior to NAC). The relapse-free survival (RFS) rates were compared among subgroups. pCR was achieved in 93 patients (20.4 %). In patients with non-pCR, the median reduction rate of Ki-67 was 60 %. A total of 15 % of patients were in the High-reduction, 63 % in the Low-reduction, and 22 % in the Increase group. The median follow-up period was 64.5 months. The 5-year RFS rates among the three groups were significantly different ($p < 0.0001$), and the differences were also observed in the HER2 ($p = 0.033$), triple-negative ($p = 0.034$), and luminal-like subtypes ($p = 0.001$). Patients in the High-reduction group showed comparable RFS to that of patients with pCR ($p = 0.363$). In patients with non-pCR, the reduction rate of Ki-67 after NAC significantly predicted RFS regardless of cancer subtypes. Therefore, patients who are non-pCR but who achieve a high reduction of Ki-67 can be expected to have a favorable prognosis similar to that of patients with pCR.

Keywords Breast cancer · Neoadjuvant chemotherapy · Ki-67 · Pathological complete response · Prognostic factor

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Introduction

Neoadjuvant chemotherapy (NAC) is now well established as the standard treatment for patients with operable and locally advanced breast cancer [1, 2]. NAC followed by surgery has several clinical benefits compared with surgery alone, for example, higher rate of breast-conservation due to tumor shrinkage and favorable long-term survival outcomes, such as recurrence-free survival (RFS) and overall survival (OS) [3]. In addition, a useful advantage of NAC related to future treatment decisions could be a confirmation of in vivo chemo-sensitivity in individual patients. Biological and pathological analysis differences between biopsy samples before NAC and surgical specimens after NAC could provide new information on predictive and prognostic markers.

A pathological complete response (pCR) after NAC is established as a powerful surrogate marker for long-term oncological outcomes [4, 5]. The achievement of pCR was selected as the primary endpoint of many NAC trials. However, the achievement rate of pCR is usually small, generally reported in only 10–30 % of patients, with standard NAC regimens [3, 6, 7]. For residual patients with

non-pCR, a surrogate marker for survival has not yet been well established. Thus, development of a useful marker for prognosis in patients with non-pCR is highly desirable.

Ki-67 is a nuclear protein with nuclear function that is expressed in all phases of the cell cycle, except G0 [8]. Ki-67 is one of the major markers of tumor proliferation, assessed by immunohistochemistry (IHC) with the anti-Ki-67 antibody called MIB-1 [9]. Many investigations have reported that Ki-67 is an independent predictive and prognostic marker in patients with operable breast cancer [10, 11]. Thus, assessment of Ki-67 is already introduced into daily practice in order to discriminate breast cancer subtypes, predict oncological outcomes, or decide on indications for adjuvant treatment [12].

In the NAC setting, changes of the absolute values of Ki-67 between pre- and post-NAC are often observed [13–15]. Reduction of the absolute value of Ki-67 after NAC compared with the value prior to NAC has been reported to be associated with a favorable prognosis [15–17]. In addition, we previously reported that the significance of this association was dependent on breast cancer subtypes [18]. However, almost all previous investigations, including ours, simply divided patients into two subgroups according to Ki-67 changes, such as Ki-67 increase or Ki-67 decrease. Also, a reduction of the absolute value of Ki-67 after NAC was commonly observed in patients with non-pCR, reported in 60–80 % of patients [18–20].

It is speculated that in patients with non-pCR, the reduction rate of Ki-67, such as High reduction or Low reduction, would affect their long-term oncological outcome. However, to the best of our knowledge, no published investigation has reported an association between the reduction rate of Ki-67 and the oncological outcome in detail. We hypothesized that the reduction rate of Ki-67 has a prognostic significance and has a potential to become a useful biomarker for oncological outcomes in patients with non-pCR. Therefore, we retrospectively analyzed whether the reduction rate of Ki-67 could discriminate oncological outcomes in patients with non-pCR after NAC and predict whether a prognostic significance of the reduction rate of Ki-67 was dependent on breast cancer subtypes.

Materials and methods

Patients and treatment

We retrospectively reviewed the clinical and pathological records of patients who received neoadjuvant anthracycline with or without taxane chemotherapy followed by curative surgery at the National Cancer Center Hospital East (Kashiwa, Japan) between January 2000 and December 2011. Written informed consent for treatment was obtained from all patients before treatment initiation. For inclusion in this analysis, the patients had to be in clinical stage II A to stage III C with histological confirmation of breast cancer by core needle biopsy, according to the American Joint Committee on Cancer staging (7th edition). All chemotherapy regimens were allowed for this analysis, if the chemotherapy was administered as anthracycline with or without taxane. Basically, NAC was administered in four to eight cycles. If HER2 positivity was confirmed by three plus of IHC scoring or c-erbB2 gene amplification by fluorescence in situ hybridization (FISH), then trastuzumab was administered as neoadjuvant treatment for 3 months after 2008. On the other hand, none of the patients with HER2-positive disease received neoadjuvant trastuzumab treatment from 2000 to 2008, because trastuzumab was not approved in Japan as neoadjuvant treatment until 2008.

Patients whose clinical or pathological parameters were not available were excluded from this analysis. Patients who received neoadjuvant hormonal therapy or a combination of chemo-hormonal therapy and who had not undergone curative surgery were also excluded from this analysis.

The indications for the composition of post-surgical treatment (adjuvant treatment) were based on the St. Gallen Consensus Recommendation at that time. In brief, none of the patients received additional chemotherapy after surgery (adjuvant chemotherapy). All patients who underwent breast-conserving surgery had routinely received adjuvant radiotherapy. Adjuvant radiotherapy was also administered to patients with mastectomy who had axillary lymph node metastasis. Patients whose hormonal status was ER and/or PgR positive by IHC underwent adjuvant hormonal therapy for at least 5 years. If HER2 positivity was confirmed by IHC or FISH, trastuzumab was administered as adjuvant treatment for 1 year after 2005. On the other hand, none of the patients with HER2-positive disease received adjuvant trastuzumab treatment between 2000 and 2005, because trastuzumab was not approved in Japan as adjuvant treatment until 2005.

Immunohistochemistry

IHC was routinely performed in our institution using formalin-fixed, paraffin-embedded tissue blocks with both pre-treatment core needle biopsy samples and post-treatment surgical excision specimens. Immunohistochemical staining of tumors for ER (Confirm anti-ER (SP1), rabbit monoclonal antibody, Ventana Medical Systems), PgR (Confirm anti-PGR (1E2), rabbit monoclonal antibody, Ventana Medical Systems), and HER2 (Pathway anti-HER2 (4B5), rabbit monoclonal antibody, Ventana Medical Systems) were performed using the automated

Benchmark XT platform (Ventana Medical Systems) and according to the manufacturer's recommendations. For Ki-67 (Clone MIB1, Dako, Glostrup, Denmark; dilution 1:50), tumors were stained in accordance with the manufacturer's recommendation. All tumor samples were evaluated by two experienced and certificated pathologists belonging to our institution. A cutoff value of ≥1 % of positively stained nuclei was used as the definition of ER- and PgR-positive disease. HER2 protein positivity was defined as a score of 3 by IHC or as positive by FISH. The methods and procedures of IHC were unchanged through the study period.

Ki-67 expression was quantified using a visual grading system. Cells stained for Ki-67 were counted and expressed as a percentage. If the staining was homogenous, then the percentage of Ki-67 positive cells among the total number of carcinoma cells counted was determined at a magnification of 400× using an eye-piece graticule and counting 10 randomly selected fields. When hot spots, defined as areas in which Ki-67 staining was particularly prevalent, were present, pathologists assessed the whole section and recorded the overall average score. Each Immunohistochemical staining included an external control to validate the Ki-67 protein expression status of each case. Therefore, the same section was used for the external control.

The subtypes were defined by IHC of core needle biopsy samples as follows. A luminal-like subtype was defined as negative HER2 status, ER positive, and/or PgR positive. The triple-negative subtype was defined as negative HER2 status, ER negative, and PgR negative. The HER2 subtype was defined as positive HER2 status regardless of ER and PgR status.

A pathological complete response (pCR) was defined by the absence of invasive carcinoma in the primary breast tumor regardless of pathological axillary node status. And then, only the presence of residual ductal carcinoma in situ was included in the pCR.

Subgroup assignment according to Ki-67 change in patients with non-PCR

Patients with non-pCR were subdivided into three subgroups according to Ki-67 change after neoadjuvant chemotherapy as follows: High-reduction group (the absolute value of Ki-67 was reduced by >80 % compared with that prior to neoadjuvant chemotherapy), low-reduction group (the absolute value of Ki-67 was reduced by 0–80 % compared with that prior to neoadjuvant chemotherapy), and increase group (the absolute value of Ki-67 was increased compared with that prior to neoadjuvant chemotherapy).

Statistical analysis

The definition of relapse excluded local breast relapse, axillary lymph node relapse, and newly diagnosed contralateral breast cancer. The relapse-free survival (RFS) period was defined as the interval from the date of surgery to that of the first diagnosis of relapse or the last follow-up date without relapse.

Associations between prognostic factors and RFS were analyzed using Chi-square test or Fisher's exact test, where appropriate. The Cox proportional hazards model was used for the estimation of multivariate analysis. Survival distributions were estimated using the Kaplan–Meier method for RFS, and the Log-rank test was used to compare survival in different strata. All statistical tests were two sided and had a 95 % confidence interval (CI), with the level of significance established at $p < 0.05$. Statistical analyses were performed using PASW (Predictive Analysis Software) 18.0 for Windows (SPSS, IBM, Chicago, Ill., USA). This study was carried out in accordance with Declaration of Helsinki and Japanese ethical guidelines for epidemiological research. We obtained the National Cancer Center institutional review board (NCC-IRB) waiver from the NCC-IRB chairperson to conduct this study.

Results

Patient characteristics

A total of 455 patients were eligible and analyzed in this investigation. Table 1 shows the baseline characteristics of all patients. The median and mean numbers of NAC cycles were 8.0 and 6.2 (range 2–8 cycles), respectively. Over 80 % of patients received NAC with anthracycline followed by taxane, and the residual patients received only the anthracycline-containing regimen. The median Ki-67 value before NAC was 20.0 % (range 1.0–80.0 %). The patients were subdivided into three subtypes according to IHC pattern. A total of 195 patients (42.8 %) were classified as Luminal-like, 126 (27.7 %) were Triple-negative, and 134 (29.5 %) were HER2 subtype. Baseline characteristics between subtypes showed no significant differences, except for the median Ki-67 value before NAC ($p < 0.001$). More details of baseline characteristics are shown in Table 1.

Response and pathological outcome after neoadjuvant chemotherapy

pCR was observed in 20.4 % of all patients. The pCR rate was highest for HER2 (35.8 %) followed by Triple-negative (25.8 %) and Luminal-like subtypes (6.7 %), and the differences in pCR rates were significantly different between subtypes ($p < 0.001$). The residual 362 patients having non-pCR were subdivided into three subgroups according to their Ki-67 change status after NAC. Of 362 patients, 53 (14.6 %), 179 (49.5 %), and 130 patients

Table 1 Baseline characteristics

	Total (%)	Luminal (%)	TN (%)	HER2 (%)	*p*
No. of patients (%)	455 (100)	195 (42.8)	126 (27.7)	134 (29.5)	
Median age (range)	53 (25–71)	52 (25–70)	53 (28–71)	53 (31–71)	0.69
Menstrual status					0.74
Premenopausal	209 (45.9)	86 (44.1)	55 (43.7)	47 (35.1)	
Postmenopausal	246 (54.1)	109 (55.9)	71 (56.3)	87 (64.9)	
Clinical tumor status					0.16
cT1	16 (3.5)	8 (4.1)	4 (3.2)	4 (3.0)	
cT2	254 (55.8)	112 (57.4)	74 (58.7)	68 (50.7)	
cT3	96 (21.1)	35 (18.0)	23 (18.3)	38 (28.4)	
cT4	89 (19.6)	40 (20.5)	25 (19.8)	24 (17.9)	
Clinical nodal status					0.084
cN positive	308 (67.7)	125 (64.1)	87 (69.0)	96 (71.6)	
cN negative	147 (32.3)	70 (35.9)	39 (31.0)	38 (28.4)	
ER status					
Positive	235 (51.6)	193 (98.9)	0	42 (31.3)	
Negative	220 (48.4)	2 (1.1)	0	92 (68.7)	
PgR status					
Positive	194 (42.6)	165 (84.6)	0	29 (21.6)	
Negative	261 (57.4)	30 (15.4)	0	105 (78.4)	
HER2 status					
Positive	134 (29.5)	0	0	134 (100)	
Negative	321 (70.5)	195 (100)	126 (100)	0	
Median Ki-67 Pre-chemotherapy (range)					<0.001
	20.0 (1–80)	9.0 (1–14)	30.0 (2–80)	20.0 (4–70)	
Neoadjuvant chemotherapy regimen					0.45
Anthracycline → Taxane	380 (83.5)	164 (84.1)	101 (80.2)	115 (85.8)	
Anthracycline only	75 (16.5)	31 (15.9)	25 (19.8)	19 (14.2)	

(35.9 %) were in High-reduction, Low-reduction, and Increase groups, respectively. The proportions of the different subtypes in these subgroups were not significantly different ($p = 0.794$), but the median Ki-67 values after NAC were significantly different among the subtypes ($p < 0.001$). The details of response and pathological outcome after NAC are shown in Table 2.

Survival according to Ki-67 change

The median follow-up period was 64.5 months and ranged from 7 to 160 months. In the non-pCR population, disease relapse was observed in 114 patients (31.5 %) during the follow-up period. Figure 1 shows the RFS curves according to Ki-67 change in all non-pCR patients. The 5-years RFS rate was 86.2 % in the High-reduction group, 75.5 % in the Low-reduction group, and 46.9 % in the Increase group. The difference in 5-year RFS among the three subgroups was statistically significant (Log-rank $p < 0.001$). We re-analyzed the 5-year RFS separately in relation to subtypes (Luminal-like, Triple negative, and HER2). The significant RFS differences shown above were also observed for all subtypes, such as Luminal-like (Log-rank $p = 0.003$, Fig. 2), Triple-negative (Log-rank $p = 0.040$, Fig. 3), and HER2 subtype (Log-rank $p = 0.034$, Fig. 4).

Figure 5 shows the RFS curves for the pCR group and the High-reduction group of non-pCR patients. There was no significant difference in the 5-year RFS between the two groups (Log-rank $p = 0.363$).

Multivariate analysis

The prognostic factors that have been well established by previous investigations and Ki-67 change status were analyzed for association with unfavorable RFS by multivariate analysis as shown in Table 3. The multivariate analysis identified several independent prognostic factors, such as pT status (Hazard ratio (HR) 2.24, 95 % CI