Table 2 Treatment outcomes of neoadjuvant chemotherapy

	Total (%) $n = 455$	Luminal (%) $n = 195$	TN (%) $n = 126$	HER2 (%) $n = 134$	p
pCR	93 (20.4)	13 (6.7)	32 (25.4)	48 (35.8)	<0.001
non-pCR	362	182	94	86	
Subgroups according to Ki-67 change					0.794
High reduction	53 (14.6)	28 (15.4)	14 (14.9)	11 (12.8)	
Low reduction	228 (63.0)	124 (68.1)	53 (56.4)	51 (59.3)	
Increase	81 (22.4)	30 (16.5)	27 (28.7)	24 (27.9)	
Median Ki-67 post-chemotherapy (range)					<0.001
	5.0	4.0	20.0	10.0	
	(0–80.0)	(0–50.0)	(0–80.0)	(1.0–70.0)	

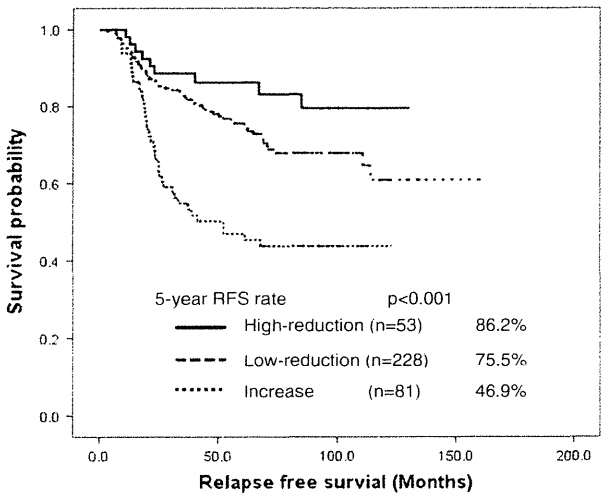

Fig. 1 RFS curves according to Ki-67 change subgroup

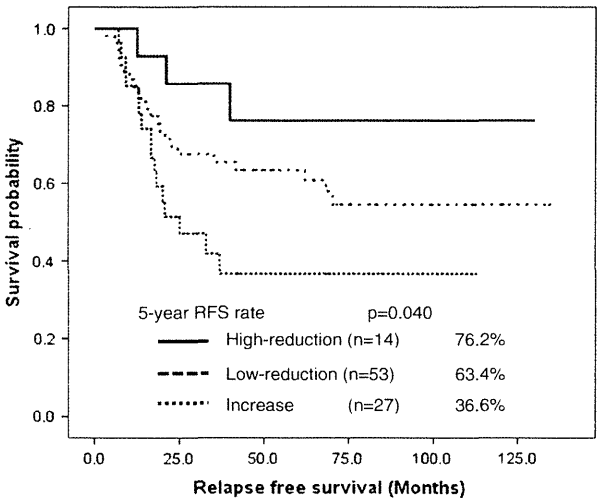

Fig. 3 RFS curves according to Ki-67 change subgroup in patients with Triple-negative subtype

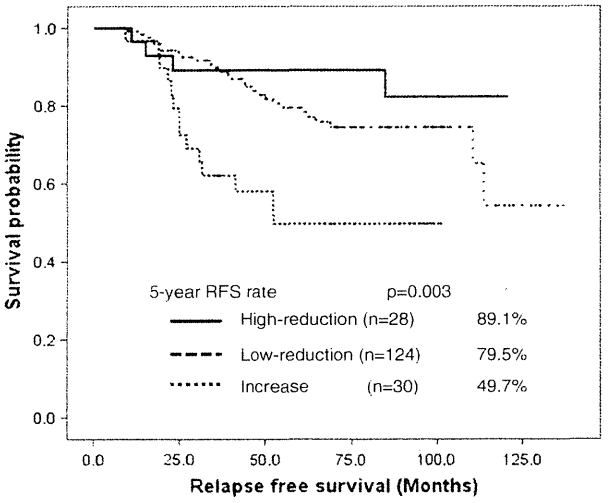

Fig. 2 RFS curves according to Ki-67 change subgroup in patients with Luminal-like subtype

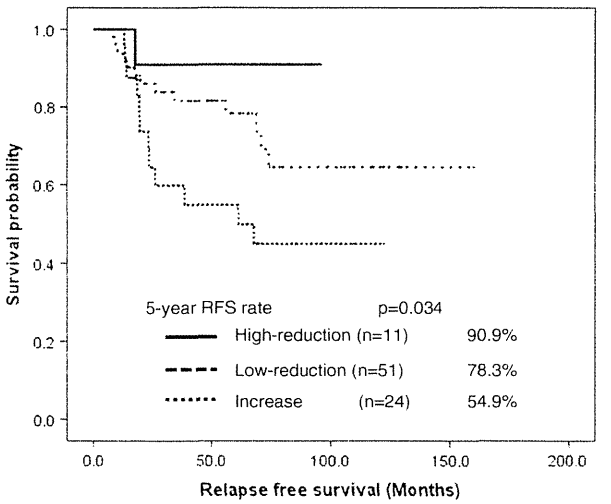

Fig. 4 RFS curves according to Ki-67 change subgroup in patients with HER2 subtype

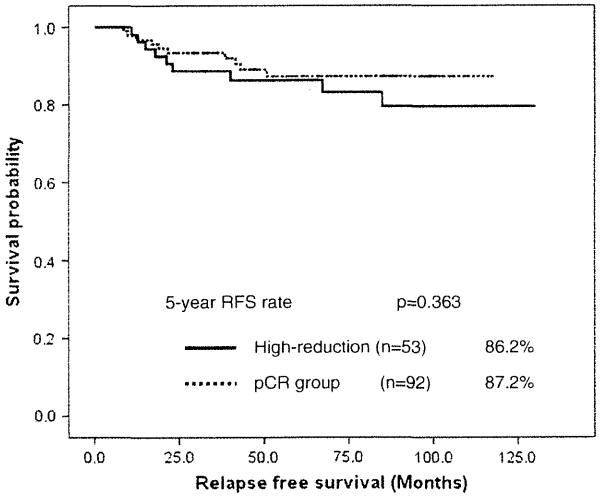

Fig. 5 RFS curves in patients with pCR and high-reduction group

Table 3 Multivariate analysis for factors related to recurrence

	Hazard ratio	95 % CI	*p* value
pT status			
pT1–2	1		
pT3–4	2.24	1.54–3.28	<0.001
pN status			
pN negative	1		
pN positive	3.51	2.16–3.28	<0.001
NAC regimen			
Anthracycline → Taxane	1		
Anthracycline only	1.24	0.79–1.93	0.339
Ki-67 changes after NAC			
High reduction	1		
Low reduction	2.18	0.94–5.06	0.071
Increase	5.26	2.23–12.4	<0.001

1.54–3.28), pN status (HR 3.51, 95 % CI 2.16–3.28), and Ki-67 increase after NAC (HR 5.26, 95 % CI 2.23–12.4).

Discussion

We assumed that in patients with non-pCR, the reduction rate of Ki-67 might affect their long-term oncological outcome. However, to the best of our knowledge, no published papers have investigated this presumption in detail. In the present investigation, we found that in patients with non-pCR after NAC, a high rate of Ki-67 reduction was associated with a better survival than that of patients who showed a low reduction or increase of Ki-67 regardless of their breast cancer subtypes. Surprisingly, in addition, patients with a high rate of Ki-67 reduction after NAC showed a comparable long-term oncological outcome to that of patients with pCR. Our findings suggested that the monitoring of changes in Ki-67 between pre- and post-NAC could be used to not only classify patients with non-pCR according to chemo-sensitivity but also to predict their survival.

Many previous investigations have shown that pCR after NAC is a powerful surrogate marker for long-term oncological outcome [4, 5]. However, it is a major drawback of this marker that the population that achieves pCR is small [3, 6, 7]. On the other hand, residual patients with non-pCR have been indiscriminately regarded as constituting a group with a high potential for disease relapse. The conventional prognostic factors, such as clinical and/or pathological response, pathological tumor size, axillary lymph node status, and histological grade, are still used to predict survival in patients with non-pCR [21, 22]. However, these conventional factors are not quite sufficient and are not satisfactory in daily practice. Another new approach, gene expression profiling, is already commercially available; however, it cannot be extrapolated to include the NAC setting because of lack of evidence in this setting. Therefore, a new biomarker that reflects the sensitivity to NAC in patients with non-pCR would be very useful and highly desirable.

We hypothesized that the reduction rate of Ki-67 has the potential to become a biomarker for survival in patients with non-pCR. Several previous investigations have already reported that changes in Ki-67 or the post-treatment absolute value of Ki-67 are associated with survival [16–19]. Also, previous investigations revealed that patients with Ki-67 reduction have better survival than those with Ki-67 increase. However, in general, a reduction of the absolute value of Ki-67 after NAC compared with that prior to NAC is widely observed, reported in 60–80 % of patients [18–20]. Nevertheless, the clinical courses in patients with non-pCR and with Ki-67 reduction are heterogeneous. The association between the reduction rate of Ki-67 and prognosis has not previously been investigated in detail. Our results revealed for the first time that the reduction rate of Ki-67 is more important for the long-term oncological outcome than a simple Ki-67 change status in patients with non-pCR.

The standard IHC methods and routine measurements of Ki-67 have not been firmly established and are now under construction [23]. Because it seems to be difficult to measure and compare the correct absolute values of Ki-67, a simple comparison of Ki-67 changes, such as High-reduction, Low-reduction, or Increase group, would be easy to introduce in clinical practice, until a reliable assay method became available. In the future, a nomogram will be constructed for patients with non-pCR to predict the

oncological outcome based on conventional prognostic factors and the reduction rate of Ki-67.

Another meaningful finding from this investigation is that RFS of patients with High reduction of Ki-67 was comparable to the RFS of patients with pCR. To the best of our knowledge, this finding is reported here for the first time. In general, patients and physicians with non-pCR are disappointed with this result of NAC treatment. However, according to our results, patients with non-pCR should not have to worry more about recurrence than those with pCR, if a high reduction of Ki-67 was achieved. Previous investigations have already reported that there were small non-pCR populations that had favorable outcomes comparable to those of pCR patients [24]. Measuring residual cancer burden, as a continuous variable derived from the primary tumor dimensions, cellularity of the tumor bed, and axillary node burden, could divide patients with non-pCR into four categories according to oncological outcome [24]. However, this method seems to be a little too intricate for daily use. On the other hand, our method of Ki-67 change might be simpler and easier to introduce in daily practice.

The patient population with High reduction of Ki-67 in our study comprised only 14.6 % of all non-pCR patients, and we acknowledge that this is a small population. There is no previous reference for the optimal cutoff level of Ki-67 reduction defined as High reduction. We analyzed the cases in this investigation using different cutoff levels for Ki-67 reduction, such as 70, 60, and 50 %, but we found that the 80 % cutoff level provided the best prognostic significance, so we adopted that. Nevertheless, in order to confirm this finding and establish the optimal cutoff level for Ki-67 reduction, further observational or validation studies with a larger sample size will be needed.

The strengths of this investigation are the following: The sample size was large, the follow-up period had adequate length, and the methods for IHC and assessment of Ki-67 were unchanged during the study period. On the other hand, there are several limitations in this investigation. It was a retrospective, single-institution analysis, there was no central pathological review of Ki-67, it included plural chemotherapy regimens, and not all patients with HER2 subtype received trastuzumab as neoadjuvant and/or adjuvant treatment. Perioperative treatment with or without trastuzumab might influence not only the Ki-67 change but also survival status. Despite these limitations, however, the simple strategy of recording Ki-67 change is useful for predicting long-term oncological outcome in patients with non-pCR.

Conclusions

This investigation revealed that the reduction rate of Ki-67 after NAC significantly predicted long-term oncological outcome in patients with non-pCR. This finding could apply to all subtypes of breast cancer. Patients who are non-pCR but who achieved a High reduction of the Ki-67 can be expected to have a favorable prognosis similar to that of patients with pCR. In order to introduce these findings into daily practice, not only larger validation studies but also optimal cutoff levels of Ki-67 reduction defined as High reduction are needed.

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Ethical standards The study was carried out in accordance with the Declaration of Helsinki and Japanese ethical guidelines for epidemiological research.

Conflict of interest The authors declare that they have no conflict of interest.

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Clinical Trial Note

Factors Affecting Enrollment in a Randomized Controlled Trial for Japanese Metastatic Breast Cancer Patients (SELECT BC-FEEL)—A Prospective Study

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To accelerate the completion of clinical trials, it is critical to obtain, at high rates, informed consent to participate from patients who are eligible. It is therefore important to know what factors affect the participation rates of eligible patients. Selection of effective chemotherapy for breast cancer and its successor selection of effective chemotherapy for breast cancer-CONFIRM are randomized controlled trials conducted for Japanese patients with chemotherapy-naïve metastatic breast cancer. These trials are intended to compare the current standard chemotherapeutic regimens in the first-line setting, which are taxanes for selection of effective chemotherapy for breast cancer and anthracyclines for selection of effective chemotherapy for breast cancer-CONFIRM, respectively, and an oral regimen of TS-1 in terms of overall survival. We are conducting prospective studies to identify the factors affecting the rates at which informed consent is obtained in selection of effective chemotherapy for breast cancer and breast cancer-CONFIRM. We are using a self-administered questionnaire that we have developed to collect information regarding patients' characteristics and attitude to clinical trials.

Key words: metastatic breast cancer – prospective cohort study – enrollment – informed consent

INTRODUCTION

A large number of clinical trials have been conducted, or are now going on, and more trials will be done in the future. Researchers who are or will be contributing ongoing or future trials are expected to enhance the speed of recruitment of eligible patients to these trials because of the rapidly intensifying competition between pharmaceutical companies or trial groups, especially in oncology fields. To accelerate trial completion, it is critical to obtain, at high rates, informed consent to participate in them from eligible patients.

Knowing what factors affect the participation rates of eligible patients is therefore important. Several studies have been done in foreign countries to address this issue (1–6). Although factors regarding physicians, patients and trial designs themselves were investigated in those studies, few of them focused on specific cancers, such as breast cancer (4–6). Since almost all patients with breast cancer are female, the factors reported to affect participating rates in these previous studies might not be applicable to trials conducted in breast cancer patients. The participating rates might depend on race, country and cultural background. Therefore, it is worthwhile to investigate the factors affecting the participating rates in trials that are conducted for Japanese breast cancer patients.

SELection of Effective Chemotherapy for Breast Cancer (SELECT BC) and its successor SELECT BC-CONFIRM are randomized controlled trials for Japanese patients with chemotherapy-naïve metastatic breast cancer. Enrollment for SELECT BC is completed, while patients are still being recruited for SELECT BC-CONFIRM. These trials are

intended to compare the current standard chemotherapeutic regimens in the first-line setting, which are taxanes for SELECT BC and anthracyclines for SELECT BC-CONFIRM, respectively, and an oral regimen of TS-1 in terms of overall survival. SELECT BC-FEEL and SELECT BC-FEEL II are accompanying studies to SELECT BC and SELECT BC-CONFIRM, respectively. SELECT BC-FEEL and SELECT BC-FEEL II were performed to identify factors affecting the rates of informed consent obtained from Japanese patients with metastatic breast cancer. We used a self-administered questionnaire to collect information on patients' characteristics and attitude to clinical trials.

DIGEST OF THE STUDY PROTOCOL

Purpose

To clarify factors that hinder participation in randomized controlled trials of Japanese patients with metastatic breast cancer by using a self-administered questionnaire.

Primary Outcome

Factors Relating to Clinical Trial Participation

Patients. All patients who were considered to be eligible for SELECT BC or SELECT BC-CONFIRM trials were asked to take part in SELECT BC-FEEL and SELECT BC-FEEL II, respectively. Eligibility criteria of SELECT BC and SELECT BC-CONFIRM trials are shown below:

(i) A histologically confirmed diagnosis of breast cancer
(ii) One of the following conditions has to be met for a diagnosis of metastatic breast cancer:
  (a) Distant metastasis at presentation.
  (b) Breast cancer that has worsened or recurred in association with distant metastasis after treatment (after surgery and pre-operative and post-operative treatment); however, local recurrence is excluded.
(iii) The presence of at least one assessable lesion. Sites treated by radiotherapy are not considered assessable lesions.
(iv) No chemotherapy with anticancer drugs after the diagnosis of metastatic breast cancer.
(v) Age of 20–75 years.
(vi) Performance status of 0–1 according to the ECOG scale.
(vii) Meeting either of the following conditions concerning previous treatment with taxane derivatives (paclitaxel or docetaxel) or anthracyclines:
  (a) No previous administration.
  (b) If such drugs were administered as pre-operative or post-operative adjuvant chemotherapy, elapse at least 6 months (168 days, 24 weeks) since the final day of treatment.
(viii) Meeting either of the following conditions concerning history of treatment with oral 5-fluorouracil derivatives:
  (a) No previous administration.
  (b) If such drugs were administered as pre-operative or post-operative adjuvant chemotherapy, elapse of at least 6 months (168 days, 24 weeks) since the final day of treatment.
(ix) Meeting both of the following conditions concerning preceding treatment:
  (a) Hormone therapy: at least 7 days have elapsed since the final day of drug treatment (irrespective of the details of treatment).
  (b) Radiotherapy: at least 14 days have elapsed since the final dose of radiation.
(x) Resistance to hormone therapy, as defined by any of the following:
  (a) Negative findings of estrogen receptor or progesterone receptor on examination of the primary lesion or recurrent lesion(s). If both the primary lesion and recurrent lesion(s) are examined and the results differ, the results for the recurrent lesion(s) will apply.
  (b) Hormone therapy is ineffective after recurrence.
  (c) Recurrence occurs during post-operative adjuvant hormone therapy or within 6 months after the final dose.
(xi) Meeting all of the following conditions regarding organ function (within 21 days before registration):
  (a) Neutrophil count (stab cells + segmented cells) of $1500/mm^3$ or higher, or white blood cell count of $3000/mm^3$ or higher.
  (b) Platelet count of $100\,000/mm^3$ or higher.
  (c) Total bilirubin concentration of not more than 2.5 times the upper limit of normal at the laboratory where the test was performed.
  (d) Aspartate aminotransferase (AST, GOT) and alanine aminotransferase concentrations (ALT, GPT) of $\leq$2.5 times the upper limit of normal at the laboratory where the test was performed.
  (e) Serum creatinine concentration of not more than the upper limit of normal at the laboratory where the test was performed.
(xii) Meeting at least one of the following conditions for cardiac function.
  (a) No cardiac disease: absence of fatigue, palpitations, shortness of breath and anginal pain during daily activities as confirmed by interview.
  (b) Cardiac disease is present, but exercise restriction is not required and the absence of fatigue, palpitations, shortness of breath and anginal pain during daily activities can be confirmed and is expected to be maintained during treatment.
(xiii) Written informed consent obtained directly from the subject.

Informed consent included in the above inclusion criteria (xiii) is not applied to participation refusers.

METHODS

A study-specific self-administered questionnaire is used to obtain information. This questionnaire was created through the processes described below.

First, a preliminary questionnaire was created. Although ideally all items and factors that had been investigated in the questionnaires used in similar previous studies in the various areas of oncology should have been adopted in our questionnaire, we limited the items to those most likely to be important in order to minimize the burden on patients and avoid possible selection bias. The following items remains.

Physician recommendation (3–6), family or friend recommendation (3,4,6), amount of explanation about the trial from the doctor or Clinical Research Coordinator (CRC) (3,4,6), agents used (3,4,6), benefit to others (5–8), relationship to the doctor (3,5), attitude toward random assignment (3–6), potential adverse effects (3,4,6,7), burden on the patient (4,6) and understanding of the concept of the trial (4,7).

Moreover, we asked the subjects who took the pre-test to rank the items shown above in the order of importance in their decision-making. Age and status of previous chemotherapy were included among the items for understanding the patients' background. Time spent to decide whether or not to participate, the decision-making process patients favor and whether or not they want to know other patients' questions to the doctors were also included. We then asked them to rank the three top items that affected their decision-making. Since the members of our quality-of-life study committee advised us to add an item regarding privacy, it was also included.

Pre-tests to evaluate the validity of the preliminary questionnaire were conducted twice. The first one was performed with 16 CRCs to modify the preliminary questionnaire. The second pre-test was done in a test-retest fashion, also with 16 CRCs, using the questionnaire modified in accordance with their advice. The time interval between the test and re-test was 1 week. The final version of the study-specific questionnaire was developed based on the concordance rates and weighed κ coefficients in the pre-tests. It included the following items.

Physician recommendation (B), family or friend recommendation (C), amount of explanation about the trial, both from the doctor or CRC (D) and the printed matter (E), agents used (F), understanding of the concept of the trial (G), benefit to others (H), relationship to the doctor (I), attitude toward random assignment (J), concerns about privacy (K), burden on the patient (L) and potential adverse effects (M).

THE ACTUAL QUESTIONNAIRE IS SHOWN IN THE APPENDIX

PROCEDURES OF THE QUESTIONNAIRE SURVEY

All patients asked to participate in SELECT BC or SELECT BC-COMFIRM are handed the study-specific questionnaire by the doctor in charge or a CRC. The patients are asked to complete the questionnaire within a short time after they decide whether or not to participate. And they are requested to hand the completed questionnaire back to the CRC. The completed questionnaires are collected within 1 month after patients inform the doctor of their decision. The CRCs who collect the questionnaires confirm that the envelopes including the questionnaires are sealed and then put the names and addresses of the hospitals and the names of the CRCs in charge on the backside of the envelopes, and send them to our data center located in Tokyo by mail.

When patients refuse to accept the questionnaires, the CRCs put the blank questionnaires into the envelopes and send them to the data center by mail. When the patients accept the questionnaire but then do not reply by 1 month after receiving the questionnaire, the CRCs do the same thing.

STATISTICAL ANALYSIS

A previous similar study showed that the odds ratios of agreement or disagreement to each statement depending on whether

Table 1. Statistical powers

Sample size	Decliners : participants	Exposure rates of participants	Statistical powers (%)
40	1 : 1	0.3	68.9
		0.5	61.6
		0.7	42.7
	1 : 2	0.3	62.8
		0.5	58.9
		0.7	44.7
	2 : 1	0.3	63.1
		0.5	52.5
		0.7	31.1
60	1 : 1	0.3	86.0
		0.5	79.7
		0.7	59.3
	1 : 2	0.3	81.9
		0.5	76.9
		0.7	60.0
	2 : 1	0.3	82.0
		0.5	73.4
		0.7	48.5
80	1 : 1	0.3	94.2
		0.5	90.1
		0.7	72.1
	1 : 2	0.3	90.9
		0.5	86.5
		0.7	70.3
	2 : 1	0.3	91.0
		0.5	84.9
		0.7	61.4

the person consented or declined to participate in a trial were >5 or <0.2 in 6 of 16 items in the questionnaire used (3).

When we assume an odds ratio of 5, a sample size 40–80, a ratio of participants to decliners ranging from 1 : 2 to 2 : 1, an exposure rate ranging from 0.3 to 0.7, and two-sided α error of 5%, the statistical power will be as shown in Table 1. The sample size of this study was therefore set at 80, but if possible more patients should be recruited in order to increase the statistical power.

Patients are divided into two groups depending on their decision of whether or not to participate in this trial, and the responses to each question are categorized and tallied. To investigate the differences in the distribution between the two groups, histograms are created of the frequency of responses to all of the questions.

To investigate the associations between patients' decision of whether or not to participate in this trial and their responses to each question, two-sided P values are calculated in the χ^2 test for dual choice questions, the Mann–Whitney test for questions using three-point or five-point Likert scales, and the t-test for questions with continuous variables. Exploratory investigations with multistep variables replaced by dichotomous variables are also done as appropriate.

To investigate the causality of each question item with respect to the decision of whether or not to participate in this trial, odds ratios and 95% confidence intervals are calculated. Stratification analysis and logistic regression analysis are also done as needed. In selecting exploratory variables in multivariate analysis, an investigation is performed using a statistical variable selection method with all question items as candidates, as well as an investigation of models of interest from previous studies and data obtained in them. We plan to conduct analyses mentioned above with the data of SELECT BC-FEEL and SELECT BC-FEEL II separately, and the combined data of those two studies.

Funding

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Conflict of interest statement

None declared.

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Appendix

(1) First please tell us your decision.

	Yes	No
(A) We would like to know if you have agreed to take part in CONFIRM trial?	☐	☐

(2) Below are some reasons that may have influenced your decision to accept or decline to take part in CONFIRM trial. Please answer each question and tick the box that shows most clearly how you feel.

	Strongly agree	Agree to some extent	Unsure	Disagree to some extent	Strongly disagree
(B) My doctor wanted me to participate in this trial.	☐	☐	☐	☐	☐
(C) My family or friends wanted me to participate in this trial.	☐	☐	☐	☐	☐
(D) I am satisfied with the explanation I received about this trial from my doctor or clinical research coordinator.	☐	☐	☐	☐	☐
(E) I am satisfied with the explanation I received about this trial from printed matter.	☐	☐	☐	☐	☐
(F) Both treatment regimens used in this trial are suitable to me.	☐	☐	☐	☐	☐
(G) I know that the trial is conducted to determine which is a better treatment.	☐	☐	☐	☐	☐
(H) I think that my participation in this trial will contribute to the benefit to future patients with the same disease.	☐	☐	☐	☐	☐
(I) I think that my relationship with my doctor will become worse, if I refuse to participate in this trial.	☐	☐	☐	☐	☐
(J) I am worried about the fact that I cannot choose which treatment to receive if I participate in this trial.	☐	☐	☐	☐	☐
(K) I am concerned about leakage of my personal information, if I participate in this trial.	☐	☐	☐	☐	☐
(L) I am concerned about an increased burden on me, if I participate in this trial.	☐	☐	☐	☐	☐
(M) I am concerned about the side effects of the treatments used in this trial, if I participate in this trial.	☐	☐	☐	☐	☐

(N) What items from (B) to (M) affected your decision most? Please rank the top three items in the order that they affected your decision.

No. 1 ( ) No. 2 ( ) No. 3 ( )

(3) We would like to know about you and your thinking. Please answer each question and tick the box that is most appropriate.

(O) What is your age?	30 or younger ☐	31–40 ☐	41–50 ☐	51–60 ☐	61 or older ☐
(P) Have you ever received chemotherapy?		Yes ☐	No ☐		
(Q) How many days did you spend to make your decision after you had received the explanation about the trial?	1 day ☐	3–4 days ☐	7 days ☐	10–14 days ☐	15 days or more ☐
(R) How do you prefer that the decision about your participation is made?	My doctor makes the decision alone. ☐	My doctor makes the decision after he or she confers with me. ☐	My doctor and I make the decision together. ☐	I make the decision after I consult my doctor. ☐	I make the decision alone. ☐

(S) Do you want to know what questions other patients who received the explanation about this trial asked to their doctors?	Strongly agree ☐	Agree to some extent ☐	Unsure ☐	Disagree to some extent ☐	Strongly disagree ☐

(4) Please rank the following items.

(T) What side effects of chemotherapy are you concerned about most? Rank the top three according to the order of most serious concern.

1. Myelosuppression (becoming more susceptible to infectious diseases)
2. Hair loss
3. Fatigue
4. Joint and/or muscle pain
5. Peripheral neuropathy (sensory change or numbness of hands and/or feet)
6. Nausea, vomiting or loss of appetite
7. Digestive symptoms (diarrhea, constipation etc.)
8. Other(s) ( )

No. 1 ( ) No. 2 ( ) No. 3 ( )

(U) Whose opinions most affected your decision on whether or not to participate in this trial? Rank the top three according to the order of importance.

1. Your parent(s)/your brother(s)/your sister(s)
2. Your spouse/child(ren)
3. Your friend(s)
4. Your doctor
5. Doctor(s) other than your doctor
6. Nurse(s)/CRC(s)
7. Other patients who participated in the trial
8. Other(s) ( )

No. 1 ( ) No. 2 ( ) No. 3 ( )

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CLINICAL TRIAL

Anastrozole versus tamoxifen as adjuvant therapy for Japanese postmenopausal patients with hormone-responsive breast cancer: efficacy results of long-term follow-up data from the N-SAS BC 03 trial

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Abstract Aromatase inhibitors are superior to tamoxifen as adjuvant therapy in postmenopausal patients with hormone-responsive breast cancer. We report the follow-up efficacy results from the N-SAS BC 03 trial (UMIN CTRID: C000000056) where anastrozole was compared with tamoxifen as adjuvant therapy in postmenopausal Japanese patients with hormone-responsive early breast cancer. The full analysis set contained 696 patients (anastrozole arm, $n = 345$; tamoxifen arm, $n = 351$). The log-rank test was used to compare the two groups in terms of disease-free survival (DFS) and relapse-free survival (RFS); Kaplan–Meier estimates were calculated. The treatment effects were estimated by Cox's proportional hazards model. To examine time-varying effect of hazard ratios, we estimated time-varying hazard ratios at time t [HR(t)] using data from time t up to 12 months. After a median follow-up of 98.5 months, hazard ratios (95 % CIs) were 0.90 (0.65–1.24; log-rank $p = 0.526$) for DFS and 0.83 (0.56–1.23; log-rank $p = 0.344$) for RFS. Hazard ratios (95 % CIs) for DFS and RFS up to 36 months were 0.69 (0.40–1.17) and 0.54 (0.27–1.06) and those after 36 months were 1.06 (0.70–1.59) and 1.05 (0.64–1.73), respectively. Time-varying hazard ratios for both DFS and RFS showed that hazard ratios were initially in favor of anastrozole and approached 1.0 at around

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36 months. Superior efficacy of anastrozole to tamoxifen suggested by the initial analysis was not confirmed in the present analysis after a long-term follow-up period. Advantage of anastrozole was the greatest immediately after switching from tamoxifen and then decreased thereafter.

Keywords Breast cancer · Adjuvant therapy · Hormonal therapy · Anastrozole · Tamoxifen

Background

Aromatase inhibitors are superior to tamoxifen as adjuvant therapy in postmenopausal patients with hormone-responsive breast cancer in terms of disease-free survival [1–7]. Most clinical trials have been conducted in western countries, and there has been concern regarding possible ethnic differences in the efficacy as well as toxicity of these hormonal agents. The cytochrome P450 (CYP) 2D6 genotype, which metabolizes tamoxifen into its more potent metabolite, endoxifen, and the distribution of the CYP19 gene (aromatase) polymorphisms differ among the Caucasian and Asian population [8]; this may potentially cause differences in the efficacy and safety of aromatase inhibitors between the Caucasian and Japanese populations. Nevertheless, our previous analysis of the N-SAS BC03 study at a median follow-up of 42 months had shown that switching from tamoxifen to anastrozole was probably to decrease disease recurrence when compared with the use of tamoxifen in postmenopausal Japanese patients with breast cancer at a magnitude similar to that observed in the western studies. Notably, we found ethnic differences in major adverse events, which may be attributable to a low baseline risk of such events in Japanese women [9].

The Oxford overview meta-analysis found that approximately 15 % of breast cancer recurrences occurred within the first 5 years after starting tamoxifen, with an incidence of approximately 17 % over the next 10 years, and approximately 9 % of breast cancer mortality occurred within the first 5 years after starting tamoxifen, with an incidence of approximately 18 % over the next 10 years. Thus, most breast cancer recurrences and deaths occurred after 5 years of tamoxifen administration during 15 years of follow-up since diagnosis [10]. Therefore, it is of clinical importance to investigate the long-term follow-up data from a randomized controlled trial of aromatase inhibitors in postmenopausal patients with early breast cancer in Japan.

Hence, here we report the efficacy results from the long-term follow-up data from the N-SAS BC 03 trial (UMIN CTRID: C000000056), in which anastrozole was compared with tamoxifen in postmenopausal patients with hormone-responsive early breast cancer who had taken tamoxifen as adjuvant therapy for 1–4 years during the 5 years of treatment.

Patients and methods

Study design

The details of the original study design as well as statistical considerations have been described elsewhere [9]. In brief, this was a multi-institutional, open-label, randomized control trial designed to compare the efficacy and safety of tamoxifen with those of tamoxifen followed by anastrozole in postmenopausal women with hormone-responsive breast cancer who remained disease-free after having received tamoxifen for 1–4 years as adjuvant therapy. The subjects were randomly assigned to continue receiving tamoxifen (20 mg/day) or to switch to anastrozole (1 mg/day). The total duration of treatment was 5 years. The primary endpoints were disease-free survival (DFS) and adverse events. The secondary endpoints were relapse-free survival (RFS), OS, and the health-related quality of life (HRQOL). At the time of randomization, treatment assignments were adjusted according to the following factors: clinical stage (I, IIA, IIB/IIIA/IIIB), the number of metastases to axillary lymph nodes (0/1–3/>3), HER2 status (unknown/0, 1+, 2+/3+), tumor size (<3/≥3 cm), estrogen receptor (ER) and progesterone receptor (PR) status [ER(+), PR(+)/ER(+), PR(−)/ER(−), PR(+)], type of surgery (breast-conserving surgery/mastectomy), duration of tamoxifen administration (1.0 to <2.0 years/2.0–4.0 years), age (<60 years/≥60 years), chemotherapy (performed/not performed), and institution. Menopause in this study was defined as follows: an age of >60 years, an age of >45 years with amenorrhea for 1 year or longer without hysterectomy, or bilateral ovariectomy.

Out of a total of 706 recruited patients, 696 patients (anastrozole arm, $n = 345$; tamoxifen arm, $n = 351$) were

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Table 1 Patients' characteristics of full analysis set

	ANA $n = 345$	TAM $n = 351$	p value
Age [median (min–max)]	60 (45–77)	60 (44–82)	0.241*
Stage			0.960†
I	142 (41.2)	147 (41.9)	
IIA	126 (36.5)	125 (35.6)	
IIB	52 (15.1)	57 (16.2)	
IIIA	11 (3.2)	11 (3.1)	
IIIB	14 (4.1)	11 (3.1)	
Pathological tumor size			0.943†
<3 cm	274 (79.4)	278 (79.2)	
≥3 cm	71 (20.6)	73 (20.8)	
Nodal status			0.370†
0	203 (58.8)	211 (60.1)	
1–3	102 (29.6)	99 (28.2)	
4–9	23 (6.7)	31 (8.8)	
10-	17 (4.9)	10 (2.8)	
ER			0.932†
Positive	321 (93.0)	326 (92.9)	
Negative	24 (7.0)	25 (7.1)	
PR			0.948†
Positive	271 (78.6)	275 (78.3)	
Negative	74 (21.4)	76 (21.7)	
Type of surgery			0.872†
Breast-conserving surgery	181 (52.5)	182 (51.9)	
Mastectomy	164 (47.5)	169 (48.1)	
HER2			0.988†
0, 1+, 2+	165 (47.8)	168 (47.9)	
3+	13 (3.8)	14 (4.0)	
Unknown	167 (48.4)	169 (48.1)	
Chemotherapy			0.660†
+	164 (47.5)	161 (45.9)	
−	181 (52.5)	190 (54.1)	

* Mann–Whitney U test

† Mantel test

n (%) was shown in all items except for age

used as a full analysis set for the present report. Patients' characteristics for the full analysis set are shown in Table 1. One patient in the anastrozole arm was excluded from the protocol because she experienced recurrence before her allocated protocol treatment was initiated. The consort diagram is shown in Fig. 1.

In the present report, we intended to investigate the efficacy data (DFS and RFS) of the long-term follow-up data as well as the time-varying effect of hazard ratios by the described statistical methods. All of the following were events considered for DFS: locoregional relapse, distant metastasis, asynchronous cancer or secondary cancer (except skin basal cell cancer/spinocellular cancer and uterine intraepithelial cancer), and death from any cause. The data were censored on the day when the above events were last confirmed to be absent or on the day when survival was last confirmed for survivors. Events for RFS were locoregional relapse and distant metastasis. The data were censored on the day when the above events were last confirmed to be absent, on the day of occurrence of asynchronous or secondary cancer, or on the day when none of the above events was confirmed. For patients who died with none of the above events confirmed, data were censored on the date of death.

Statistical methods

For each treatment group, backgrounds were summarized and compared with the Mantel test. The log-rank test was used to compare the two groups in terms of DFS and RFS, and Kaplan–Meier estimates were calculated. The treatment effects were estimated by Cox's proportional hazards model and were expressed as hazard ratios with associated 95 % confidence intervals (CIs). To examine the time-varying effect of hazard ratios [11], we estimated time-varying hazard ratios at time t [HR(t)] using data from time t up to 12 months later. If the proportional hazards' assumption was admissible, these ratios took a nearly constant value.

Results

After a median follow-up of 98.5 months (range, 2.8–134 months), the number of the events related to DFS was 71 in the ANA arm as compared with 77 in the TAM arm; the number of events related to RFS was 46 in the ANA arm as compared with 54 in the TAM arm. The unadjusted hazard ratio was 0.90 (95 % CI 0.65–1.24; log-rank $p = 0.526$) for DFS and 0.83 (95 % CI 0.56–1.23; log-rank $p = 0.344$) for RFS. Kaplan–Meier prevalence curves for DFS and RFS are shown in Figs. 2 and 3, respectively. The distribution of the events is shown in Table 2.

The estimated hazard ratio (95 % CIs) for DFS and RFS until each time point (administrative right censored data) was as follows: until 24 months, 0.46 (0.23–0.91) and 0.47 (0.21–1.05); until 36 months, 0.69 (0.40–1.17) and 0.54 (0.27–1.06); until 48 months, 0.75 (0.48–1.19) and 0.60 (0.34–1.04); until 60 months, 0.82 (0.55–1.24) and 0.69 (0.42–1.14); until 72 months, 0.87 (0.59–1.28) and 0.78 (0.49–1.24); until 84 months, 0.96 (0.68–1.36) and 0.93 (0.61–1.42); until 96 months, 0.89 (0.64–1.25) and 0.87 (0.58–1.31); until 108 months, 0.87 (0.63–1.21) and 0.84 (0.56–1.25); and until 120 months: 0.89 (0.64–1.23) and 0.83 (0.56–1.23), respectively. Hazard ratios (95 % CIs)

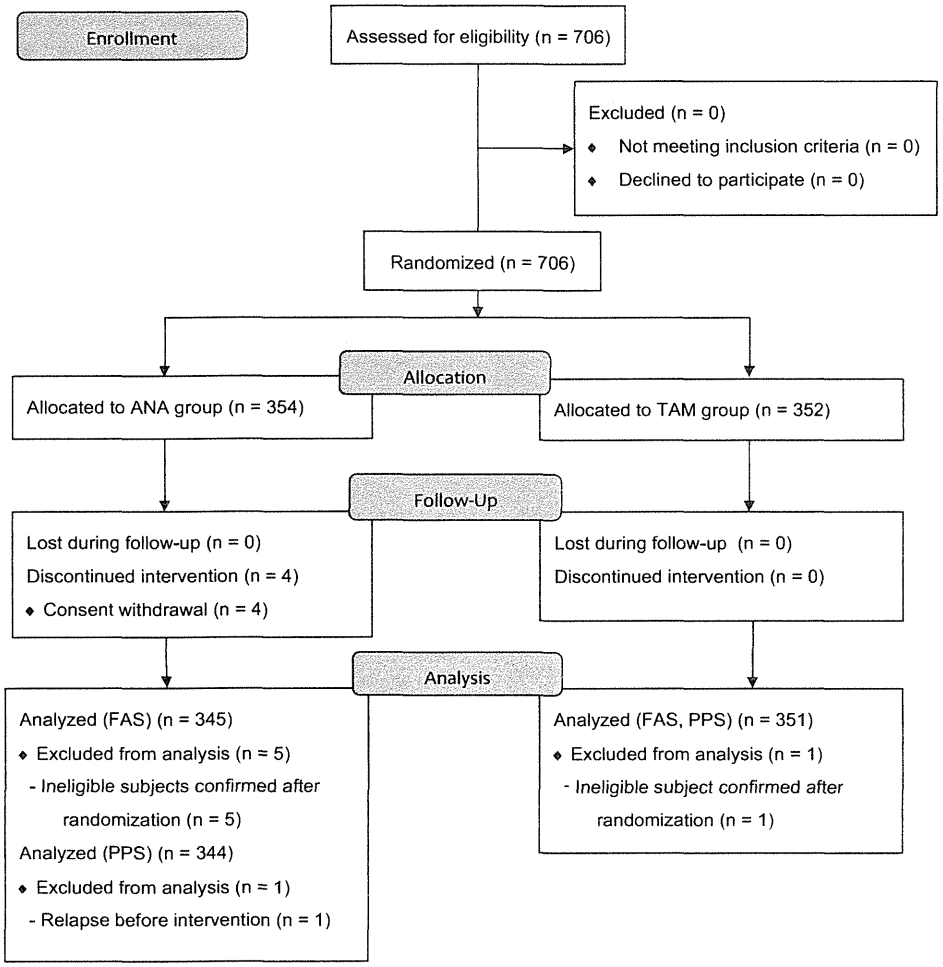


Fig. 1 Consort diagram. *ANA* anastrozole arm, *TAM* tamoxifen arm, *FAS* full analysis set (Patients randomly assigned and started protocol treatment), *PPS* per protocol set (Patient fulfilled the protocol in terms of the eligibility, interventions, and outcome assessment)

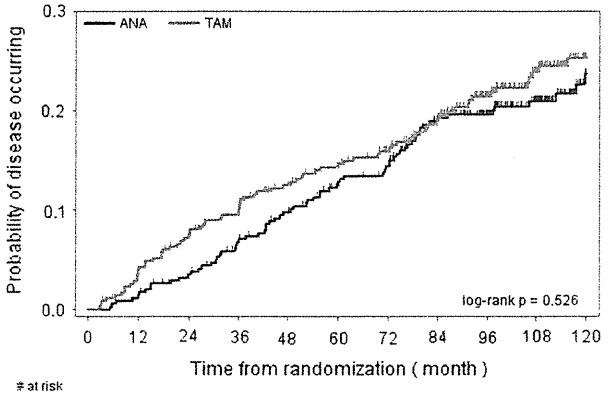
for DFS and RFS until 36 months were 0.69 (95 % CI 0.40–1.17) and 0.54 (95 % CI 0.27–1.06), and those after 36 months were 1.06 (95 % CI 0.70–1.59) and 1.05 (95 % CI 0.64–1.73), respectively. Time-varying hazard ratios for DFS and RFS for anastrozole versus tamoxifen that were described in Figs. 4 and 5, respectively, showed that both of hazard ratios were initially in favor of anastrozole and then approached 1.0 at around 36 months.

No significant difference in the total number of the patients who died of any cause (15 in ANA arm and 22 in TAM arm) was observed between the groups (log-rank $p = 0.210$).

Discussion

The superior efficacy of anastrozole over tamoxifen in Japanese postmenopausal patients with hormone-responsive early breast cancer was suggested by the N-SAS BC03 trial at a median follow-up of 42 months [9]. The number of events included in the previous report was small; therefore, we attempted to investigate if the superior efficacy of anastrozole in the Japanese population could be confirmed by long-term follow-up data, which could accumulate a larger number of events. Although the number of the total events increased from 63 to 148 after a median follow-up of 98.5 months, the hazard ratio of anastrozole over tamoxifen for DFS and RFS increased. When we looked at the data closely, both hazard ratios for DFS and RFS were in favor of anastrozole before 3 years, but they increased to 1.0 after around 3 years; this corresponded to the median treatment period of the protocol therapy. A similar number of events occurred in both arms after cessation of the protocol treatment, and this may have canceled the superior efficacy of anastrozole, which had been suggested during the protocol treatment.

Similar trends in the hazard ratio during the follow-up period have been observed in other clinical trials, which



at risk

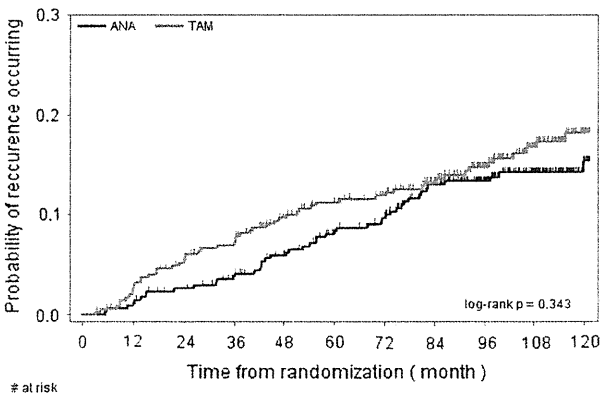
ANA	344	338	327	312	299	283	274	242	204	144	64
TAM	351	335	319	308	286	274	261	237	188	135	75

Log-rank test: $p = 0.526$

	HR	(95% CI)	p-value
Allocation (ANA/TAM)	0.90	(0.65–1.24)	0.526
Allocation (ANA/TAM) before 36 months	0.69	(0.40–1.17)	0.169
Allocation (ANA/TAM) after 36 months	1.06	(0.70–1.59)	0.784

ANA = anastrozole, TAM = tamoxifen

Fig. 2 Kaplan–Meier prevalence curves for disease-free survival



at risk

ANA	344	339	330	320	307	292	283	254	216	152	70
TAM	351	338	323	314	291	279	267	244	197	142	78

Log-rank test: $p = 0.343$

	HR	(95% CI)	p-value
Allocation (ANA/TAM)	0.83	(0.56–1.23)	0.344
Allocation (ANA/TAM) before 36 months	0.54	(0.27–1.06)	0.072
Allocation (ANA/TAM) after 36 months	1.05	(0.64–1.73)	0.835

ANA = anastrozole, TAM = tamoxifen

Fig. 3 Kaplan–Meier prevalence curves for relapse-free survival

compared sequential aromatase inhibitors versus tamoxifen for 5 years. For instance, the hazard ratio of exemestane over tamoxifen in the IES trial at the 30.6-month report was 0.68 [12], at the 55.7-month report was 0.76 [13], and at the 91.0-month report was 0.81 [7]. This trend for the hazard ratio over time does not simply mean that the efficacy of aromatase inhibitors is negated after cessation of treatment.

Table 2 Distribution of the events

Event	ANA ($n = 347$)	TAM ($n = 349$)
Local recurrence	13	20
Distant recurrence	28	31
Primary cancer in the contralateral breast	4	4
Intercurrent death	6	3
Second primary non-breast cancer	20	19
Total	71	77

ANA anastrozole, *TAM* tamoxifen

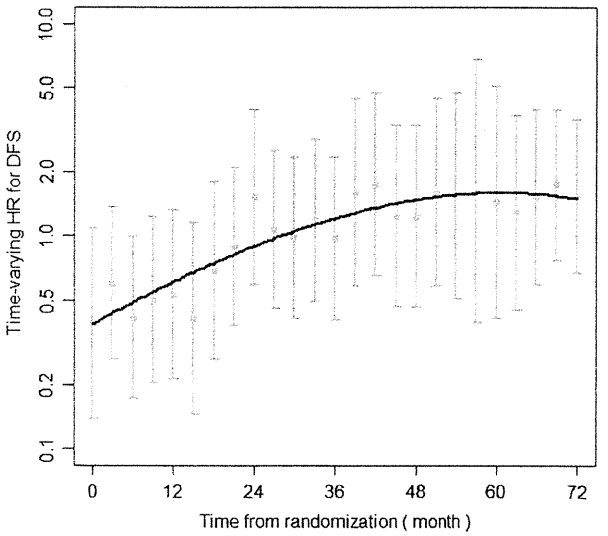


Fig. 4 Time-varying hazard ratios for disease-free survival for anastrozole versus tamoxifen. *Gray lines* show hazard ratio and 95 % confidence interval of each 12 months period and a curve shows cubic-smoothed point estimation of the hazard ratio

Because tamoxifen has retained superior efficacy over no treatment or placebo even after the completion of the 5-year treatment period, which is the so-called "carry-over effect" [10], anastrozole may also have a similar effect as tamoxifen. Some clinical trials have shown that 10 years of hormonal treatment has superior efficacy to 5 years of treatment [14, 15]. Therefore, anastrozole treatment for longer than 5 years could result in better outcomes. Clinical trials evaluating this are ongoing including N-SAS BC 05, which our group conducted (UMIN CTRID: 000000818).

As for overall survival, the total number of the patients who died of any cause was 15 in the ANA arm and 22 in the TAM arm, which did not reach a statistically significant level (log-rank $p = 0.210$). Similar to most other clinical trials that have compared aromatase inhibitors with tamoxifen, even after long-term follow-up, the number of events in this study was so low that we could not conclude whether anastrozole was more efficacious than tamoxifen

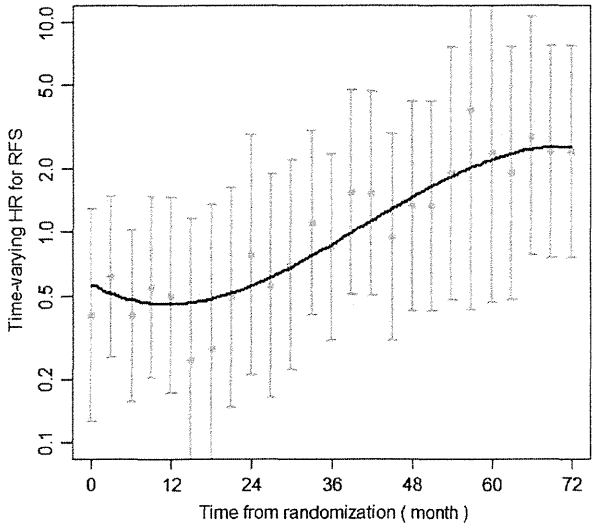


Fig. 5 Time-varying hazard ratios for relapse-free survival for anastrozole versus tamoxifen. *Gray lines* show the hazard ratio and 95 % confidence interval of each 12 months period; a curve shows cubic-smoothed point estimation of the hazard ratio

in terms of overall survival. A meta-analysis of clinical trials would bring conclusive results.

In summary, the efficacy of sequencing from tamoxifen to anastrozole over tamoxifen alone in the Japanese population was investigated over a long-term follow-up period through the N-SAS BC03 trial. The efficacy was the greatest immediately after switching from tamoxifen and then decreased thereafter.

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Appendix

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ORIGINAL ARTICLE

Evaluation of HER2-based biology in 1,006 cases of gastric cancer in a Japanese population

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Abstract
Background The ToGA trial demonstrated the beneficial effect of trastuzumab in gastric cancer patients with human epidermal growth factor receptor 2 (HER2)-overexpressing tumors. Therefore, evaluation of the relationship between HER2 expression and gastric cancer biology using a validated system has become an even more important task. Herein, we verified the correlation between HER2 overexpression in the tumor and the clinical course of gastric cancer patients.
Methods A total of 1,006 consecutive patients with gastric cancer who underwent surgery at the National Cancer Center Hospital East between January 2003 and July 2007 were examined using the tissue microarrays approach. HER2 expression was determined based on an immunohistochemistry score of 3+, or an immunohistochemistry score of 2+ plus HER2 gene amplification as detected by double-color fluorescent in situ hybridization. A retrospective review of the medical records was conducted to determine the correlation between the presence of HER2 overexpression and clinicopathological factors. Then, in 948 patients who had undergone curative resection, HER2 status was compared with the survival.
Results HER2 overexpression was detected in 118 (11.7 %) patients. HER2 overexpression was correlated with age, gender, grade of differentiation, expanding growth pattern, and nodal status. In the survival analysis, HER2 overexpression was not found to be correlated with either disease-specific survival or recurrence-free survival.
Conclusions HER2 overexpression in the tumor was not identified as a significant prognostic factor in patients with operable gastric cancer. The HER2-targeted therapy may be beneficial in a proportion of cases.

Keywords Gastric cancer · HER2

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Introduction

Overexpression of the human epidermal growth factor receptor 2 (HER2) protein has been detected in various cancers [1]. With regard to breast cancer, approximately two decades have elapsed since HER2 was functionally implicated in the pathogenesis. At present, an HER2-based concept of tumor biology has been established, and trastuzumab (Herceptin, Genentech/Roche), a monoclonal humanized antibody directed against HER2, is a pivotal agent for the management [2]. In gastric cancer also, many publications have suggested a similar role of HER2 [3–8]. However, validated methods and scoring systems for evaluation of the HER2 status have remained inconsistent. This discordance has been attributed to the heterogeneous patterns of expression and incomplete membrane immunoreactivity in HER2-positive gastric cancer cells. Therefore, the clinical significance and prognostic value of HER2 have remained controversial. Recently, a new assessment of the HER2 scoring system for gastric cancer was proposed by Hofmann and colleagues [9], and subsequently, a

randomized international phase III clinical trial, ToGA, was performed using modified Hofmann's criteria. The trial revealed impressive benefits of the addition of trastuzumab to a cisplatin plus fluoropyrimidine chemotherapy doublet in patients with HER2-overexpressing gastric cancers [10]. Therefore, evaluation of the relationship between HER2 expression and gastric cancer biology using a validated scoring system has become an even more important task. Herein, we verified the correlation between tumor HER2 overexpression and the clinical course of gastric cancer using the tissue microarrays (TMA) approach.

Patients and methods

Patients and disease staging

A total of 1,006 consecutive patients with gastric cancer who underwent surgery at the National Cancer Center Hospital East between January 2003 and July 2007 were examined for the present study. The medical records and surgical specimens of these patients were retrospectively evaluated after obtaining approval from the Investigational Review Board of the National Cancer Center. The disease stage was determined according to the International Union Against Cancer (UICC)-TNM classification (seventh edition) [11]. Neo-adjuvant and adjuvant chemotherapy was administered in 54 (5.4 %) and 67 (6.7 %) patients, respectively.

Tissue microarray and immunohistochemistry

Representative tumor areas were selected and marked on hematoxylin and eosin (H&E)-stained slides for the construction of microarrays. Duplicate cylindrical cores with a diameter of 2.0 mm were prepared from the same tissue block for each case using a manual tissue arrayer (Azumaya Ika Kikai, Tokyo, Japan) and assembled in a tissue microarray format. Serial 4 μm sections were used for immunohistochemical staining. The reliability of tissue microarrays for the evaluation of HER2 gene amplification has been demonstrated in breast cancer [12]. Immunohistochemical staining was performed using the fully automated Ventana Benchmark ULTRA (Roche Diagnostics) device. Sections were dewaxed, then subjected to pretreatment with CC1 for 30 min. Then, sections were washed with reaction buffer followed by incubation with the rabbit monoclonal primary antibody HER2/neu (Clone 4B5; Roche Diagnostics) for 28 min. On-board detection using the ultraView Universal DAB kit (Roche Diagnostics), used in accordance with the manufacturer's recommendations, was used to detect the location of the primary antibody HER2 followed by counterstaining with hematoxylin 11 (Roche Diagnostics). Tissue from gastric cancer in which HER2 gene amplification had been detected by in situ hybridization (ISH) was used as controls.

Procedure for double-color ISH and evaluation of HER2 gene amplification

HER2 gene amplification was detected by the double-color ISH technique using the Ventana Benchmark ULTRA and a fully automated INFORM HER2 Dual ISH DNA Probe Cocktail assay (Roche Diagnostics). The sections were deparaffinized and pretreated with CC2 buffer (Roche Diagnostics) using high heat. An ISH-protease 3 (Roche Diagnostics) was added to the sections for 8 min for enzyme digestion of protein. A cocktail of an HER2 dinitrophenyl (DNP)-labeled probe and Chr17 digoxigenin (DIG)-labeled probe was dropped on to the sections, followed by incubation for 6 h. To detect the HER2 probe, the ultraView silver in situ hybridization (SISH) DNP Kit (Roche Diagnostics) was used. After the SISH signals were developed, the ultraView Red ISH DIG detection Kit (Roche Diagnostics) was used for detection of the Chr17 probe. Sections were counterstained with hematoxylin II (Roche Diagnostics) for 8 min and post counterstained with Bluing reagent (Roche Diagnostics) for 4 min.

The HER2 and Chr17 signals were counted in 40 nuclei at the hotspot by immunohistochemical staining. A discrete signal was counted as a single copy of HER2 or Chr17. A cluster was defined as numerous overlapping SISH signals in the nuclei that could not be detected individually. According to the manufacturer's guide, clusters were subdivided into small and large clusters using the size of a single signal as reference. Then, a small cluster was counted as 6 signals and a large cluster as 12 signals. The HER2 gene expression was classified as nonamplified if the HER2/Chr17 ratio was <2.0 and as amplified if the HER2/Chr17 ratio was ≥ 2.0.

Evaluation of HER2 expression

HER2 overexpression was determined using the proposed scoring scheme in the relevant subgroup analysis of the ToGA trial [10]. Immunohistochemically stained full-face sections from each of the TMA blocks were digitized using the Slide Path and the Nano Zoomer Digital Pathology (NDP) System (Hamamatsu, Welwyn Garden City, UK). Approximately 7 min were required to scan a slide at a resolution of 40×. Two individuals (M.A. and K.K.), who were blind to the clinical data, reviewed the digital images. Evaluation and scoring of the HER2 protein expression was performed according to Hofmann's criteria. This scoring system, described below, has been validated for use in

gastric cancer: 0 = staining or membrane reactivity in <10 % of cancer cells; 1+ = faint membrane reactivity in >10 % of cancer cells or cancer cells with reactivity in only a part of the cell membrane; 2+ = weak or moderate complete or basolateral membrane staining in >10 % of cancer cells; and 3+ = strong complete or basolateral membrane staining in >10 % of cancer cells [9]. An immunohistochemistry score of 3+, or immunohistochemistry score of 2+ plus HER2 gene amplification as detected by double-color fluorescent in situ hybridization, was defined as overexpression of HER2. Consequently, the patients in whom either of the duplicate cores was estimated as showing overexpression of HER2 were determined to be HER2 positive.

Survival analysis

The median (range) follow-up period of the surviving cases was 60.9 (1–105.5) months. The survival analysis was performed in 948 patients who undergone no residual cancer (R0) resection. Disease-specific survival (DSS) time in the patients was defined as the interval between the date of surgery and the date of cancer-related death or the date of last contact. The duration of recurrence-free survival (RFS) was calculated from the date of the operation to the date on which the first recurrence was diagnosed.

Statistics

The estimated HER2 scores of each case were compared in relationship to the demographics and tumor-related factors. Survivals were estimated using the Kaplan–Meier method, and differences were determined using a log-rank test. Fisher's exact test and Cramér's measure of association were used for comparison of the covariates between the patient groups with and without tumor HER2 overexpression. The Cox proportional hazard model and log-rank test were used for the univariate analysis performed to identify factors influencing disease-specific survival and disease-free survival in patients who underwent curative resection. The significance level was set at $p < 0.05$. All statistical analyses were performed using Dr. SPSS II for Windows (SPSS Japan, Tokyo, Japan).

Results

Demographic characteristics

In the 1,006 cases enrolled in the study, the median age of the patients at diagnosis was 64 years (range 18–92 years); the patients were all Japanese. The demographics and tumor-related factors are summarized in Table 1. Of the

Table 1 Patient demographics and tumor-related factors in 1,006 patients with gastric cancer

Gender, *n* (%)	
Male/female	677/329
Age, years	
Median/range	64/18–92
Histological features, *n* (%)	
Papillary	23 (2.3)
Tubular	472 (46.9)
Poorly differentiated	352 (35.0)
Signet ring cell	137 (13.6)
Mucinous	21 (2.1)
Mixed endocrine and tubular	1 (0.1)
Tumor location, *n* (%)	
Esophagogastric junction	31 (3.1)
Proximal third of stomach	221 (22.0)
Middle third of stomach	472 (46.9)
Distal third of stomach	282 (28.0)
Macroscopic tumor type, *n* (%)	
Type 0	521 (51.8)
Type 1	27 (2.7)
Type 2	118 (11.7)
Type 3	246 (24.5)
Type 4	77 (7.6)
Type 5	17 (1.7)
Pathological depth of penetration, *n* (%)	
T1[a]	505 (50.2)
T2[a]	127 (12.6)
T3[a]	232 (23.1)
T4[a]	142 (14.1)
Pathological nodal status, *n* (%)	
N0[b]	609 (60.5)
N1[b]	141 (14.0)
N2[b]	100 (9.9)
N3a[b]	88 (8.7)
N3b[b]	60 (6.0)
Nx[b]	8 (0.8)
Pathological TMN stage, *n* (%)	
Stage I	544 (54.1)
Stage II	213 (21.1)
Stage III	188 (18.8)
Stage IV	61 (6.1)
Surgical procedure, *n* (%)	
Total gastrectomy	276 (27.4)
Distal gastrectomy	650 (64.6)
Proximal gastrectomy	75 (7.5)
Partial resection of stomach	5 (0.5)
Resection margin, *n* (%)	
R0[c]	948 (94.2)
R1[c]	26 (2.6)
R2[c]	32 (3.2)