INTRODUCTION

MicroRNAs (miRNAs) have emerged as a new class of non-coding genes involved in regulating cell proliferation, differentiation and viability (1). miRNAs are single-strand small RNA molecules that are between 19 and 22 nucleotides in length. miRNAs primarily regulate gene expression through the inhibition of RNA translation by base pairing of their 'seed region', nucleotides 2–8, to the 3′ untranslated region of the target RNAs (2). miRNA can also facilitate targeting of specific mRNAs for cleavage, resulting in the down-regulation of target mRNAs (3).

Hypoxia is a common feature of pathological conditions such as tissue ischemia and inflammation, as well as solid tumors (4). Multiple hypoxic responses that impact tumorigenesis are mediated through the hypoxia-inducible factors (HIFs), composed of alpha (HIF-α) and beta (HIF-β; ARNT) subunits (4). Under hypoxia, HIF-α subunits are stabilized and heterodimerize with HIF-β in the nucleus. This heterodimeric complex binds to hypoxia response elements (HREs) and modulates the expression of multiple target genes that are important for angiogenesis, cell survival and tumorigenesis. Two HIF-α proteins, HIF-1α and HIF-2α regulate the expression of overlapping and unique target genes (4). In addition to the transcriptional activation of multiple genes, hypoxia is also involved in the regulation of miRNAs (5).

Several studies have reported that miR-210 is a highly up-regulated miRNA in hypoxic cells and have demonstrated its importance for cell survival (6–8). miR-210 was shown to be HIF-1α dependent and to have a functional role during tumor initiation (7). A recent study showed that HIF-1α could be a predominant and sufficient regulator of miR-210; however, in the presence of HIF-1α, HIF-2α could also mediate miR-210 expression (8). In addition to being one of the predominant hypoxia-inducible miRNAs, the expression of miR-210 is reported to be up-regulated in many cancers including breast (9,10), non-small-cell lung (11), head and neck (12) and pancreatic cancers (13), glioblastoma (14), malignant melanoma (8,15) and renal cell carcinoma (16). Overexpression of miR-210 was shown to indicate a poor prognosis in breast cancer patients (9,10), head and neck (12) and pancreatic cancer patients (13). miR-210 was also shown to be overexpressed at late stages of non-small-cell lung cancer (11).

Emerging data demonstrate that stratification of tumors by gene expression profiles divides breast cancer into four common subtypes that are associated with different clinical outcomes (17). Two of them are estrogen receptor (ER) positive (luminal and luminal/HER2+) and two are ER negative (basal-like and HER2 positive) (17,18). Although the immunohistochemical staining profile can be a useful surrogate for gene expression analysis, the optimal immunohistochemical profile of the basal-like subtype remains unclear. However, the basal-like category is composed almost entirely of triple-negative breast cancers (TNBCs; tumors lacking ER, progesterone receptor (PgR) and HER2 expression) (18,19). A simplified method of classification, based on immunohistochemical assays for ER, PgR and HER2, is clinically useful, and clinicians are increasingly taking triple-negative status into account in clinical decision-making and therapeutic protocol design. Triple-negative and basal-like tumors account for about 10–15% of all invasive breast cancers, and they usually have higher histological grades and more aggressive clinical behavior than hormone receptor-positive breast cancers (20,21).

In this study, we investigated the correlations between miR-210 expression, and clinicopathological parameters and prognosis in Japanese TNBC patients.

METHODS

Patients

A total of 161 surgically resected breast carcinomas with tissues available were selected from the archive of the Department of Breast and Endocrine Surgery, Nagoya City University Hospital in Japan. Specimens were obtained from patients who underwent surgery between January 1996 and July 2007. Tissues were fixed in 10% buffered formalin and embedded in paraffin and/or placed in liquid nitrogen immediately after resection and stored at −80°C until RNA extraction. Informed consent was obtained before the surgery. The histological grade was estimated according to the Bloom and Richardson method proposed by Elston and Ellis (22). Disease-free survival (DFS) was defined as the interval from the date of primary surgery to the earliest occurrence of one of the following: locoregional recurrence, distant metastasis or death from any cause. Overall survival (OS) was defined as the interval from the date of primary surgery to death from any cause. The median follow-up period was 5.4 years (range 3–149 months). This protocol was approved by the Institutional Review Board of Nagoya City University Graduate School of Medical Sciences and conformed to the guidelines of the 1975 Declaration of Helsinki.

Quantitative Reverse Transcription-PCR Detection of miRNA and mRNA

Total RNA from homogeneous microscopically confirmed breast cancer tissue was isolated from ~500 mg of each frozen specimen with TRIZOL reagent (Invitrogen Japan K.K., Tokyo, Japan) for RNA extraction according to the manufacturer's recommendations as described previously (21). cDNA was reverse transcribed from total RNA samples using specific miRNA primers from the TaqMan MicroRNA Assays and reagents from the TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). The resulting cDNA was amplified by PCR using TaqMan MicroRNA Assay primers with the TaqMan Universal PCR Master Mix and analyzed with a 7500 ABI

PRISM Sequence Detector System according to the manufacturer's instructions (Applied Biosystems) as described previously (23). The relative levels of miR-210 expression were calculated from the relevant signals by normalization with the signal for U6B miRNA expression. The assay names for miR-210 were has-mir-210 (Applied Biosystems).

Immunohistochemical Analysis

ERα and PgR protein expression status was confirmed by immunohistochemistry (IHC) as follows. One 4-μm section of each submitted paraffin block was stained first with hematoxylin and eosin to verify that an adequate number of invasive carcinoma cells were present and that the fixation quality was adequate for IHC analysis. Serial sections (4 μm) were prepared from selected blocks and float mounted on adhesive-coated glass slides, for staining with monoclonal mouse anti-human ERα antibody (1D5; DAKO, Glostrup, Denmark) at 1:100 dilution and monoclonal mouse anti-human PgR antibody (636; DAKO) as described previously (21). The DAKO Envision system (DAKO EnVision-labeled polymer, peroxidase) was used for detection. Immunostaining for epidermal growth factor receptor (EGFR) was performed using the EGFRpharmDx assay detection system (prediluted; DAKO). After the entire slide was evaluated by light microscopy, expression of ERα and PgR was scored by proportion and intensity, according to Allred's procedure (24). In brief, the proportion scores that represented the estimated proportion of tumor cells staining positive were as follows: 0 (none), 1 ($<1/100$), 2 ($\geq 1/100$, $<1/10$), 3 ($\geq 1/10$, $<1/3$), 4 ($\geq 1/3$, $<2/3$) and 5 ($\geq 2/3$). Any brown nuclear staining in invasive breast epithelium counted toward the proportion score. The intensity scores, representing the average intensity of the positive cells, were as follows: 0 (none), 1 (weak), 2 (intermediate) and 3 (strong). The proportion and intensity scores were then added to obtain a total score, which could range from 0 to 8. Tumors with a score of 0 or 2 were considered to be negative and those with a score of 3 or greater were considered to be positive for ERα expression. HER2 immunostaining was evaluated using the same method as is employed by the HercepTest (DAKO). To determine the score for HER2 expression the membrane staining pattern was assessed and scored on a scale of 0 to 3+. Tumors with scores of 0 and 1 were considered to be negative for HER2 overexpression. To determine the score for EGFR expression, the membrane staining pattern was assessed and scored on a scale of 0 to 3+ using the same method as for the HER2 scoring. Tumors with scores of 0 and 1 were considered to be negative and tumors with scores of 2 and 3 were considered to be positive for EGFR overexpression.

Statistical Analyses

All molecular and immunohistochemical analyses were performed blinded to clinical data. Statistical calculations were performed with StatView-J 5.0 software (SAS Institute, Inc., Cary, NC, USA). The Mann–Whitney *U*-test was performed for the analyses of ERα protein and miR-210 expression. The relationships between ERα protein expression or miR-210 expression, and clinicopathological factors were assessed by χ^2 and Fisher's exact probability tests. DFS and OS curves were generated by the Kaplan–Meier method and verified by the log-rank test. A Cox proportional hazards regression analysis was used for univariate and multivariate analyses of prognostic values. Differences were considered significant when a P value < 0.05 was obtained.

RESULTS

miR-210 Expression and ERα Status

We examined the correlation between miR-210 expression and ERα protein expression in 161 samples of Japanese breast cancer tissue (58 triple-negative TNBC, and 103 ER positive and HER2 negative), because miR-210 has been linked to the metastatic potential of TNBCs (10). In order to simplify the analysis, we excluded HER2-positive breast cancer from this analysis. In order to examine the relationship between miR-210 expression and ERα expression levels, we divided the ERα-positive, HER2-negative breast cancers ($n = 103$) into two subgroups: one group showed low ERα protein expression (Allred score: 3–6; $n = 42$) and another group showed high ERα protein expression (Allred score: 7 or 8; $n = 61$; Table 1). Except for tumor grade, the characteristics of the analyzed breast cancers were similar with regard to patient age, tumor size and nodal status among these two groups of ERα-positive, HER2-negative breast cancers and TNBCs (Table 1). TNBCs showed higher tumor grades than did the ERα-positive and HER2-negative breast cancers. As shown in Fig. 1, the miR-210 expression in TNBCs (median relative miR-210 expression: 6.2; average relative miR-210 expression: 11.1 ± 2.60) was significantly higher than that in both the high ERα expression tumors (median 1.39, average 2.48 ± 0.43; Mann–Whitney *U*-test, $P < 0.001$) and the low ERα expression tumors (median 1.51, average 3.15 ± 0.61; Mann–Whitney *U*-test, $P < 0.001$).

miR-210 Expression as a Prognostic Marker in Breast Cancer

Next, we examined the relationship between the expression of miR-210 in tumor specimens and patient outcome in 58 TNBCs with long-term follow-up. miR-210 expression showed a significant inverse correlation with DFS when divided at the median miR-210 expression level (log-rank test; $P = 0.046$). Then, TNBCs were divided into three groups according to miR-210 expression levels: low expression (relative miR-210 expression <4; $n = 19$), intermediate expression ($4 \leq$ relative miR-210 expression <9; $n = 19$)

Table 1. Patient and tumor characteristics in ERα-positive and HER2-negative breast cancers, and TNBCs

	Total (161)	ERα(+) HER2(−)			Triple negative ($n = 58$)	P value[a]
		Subtotal ($n = 103$)	Allred score: 3–6 ($n = 42$)	Allred score: 7, 8 ($n = 61$)		
Age (years)						
≤50	51	30	18	12	21	0.38
>50	110	73	24	49	37	
Tumor size (cm)						
≤2	42	27	10	17	15	>0.99
>2	117	74	30	44	43	
Unknown	2	2	2	0	0	
Nodal status						
Negative	95	55	25	30	40	0.12
Positive	58	41	15	26	17	
Unknown	8	7	2	5	1	
Grade						
1	21	18	9	9	3	<0.0001
2	65	49	20	29	16	
3	53	14	7	7	39	
Unknown	22	22	6	16	0	

ER, estrogen receptor.
[a]ERα (+) HER2(−) vs. triple negative.

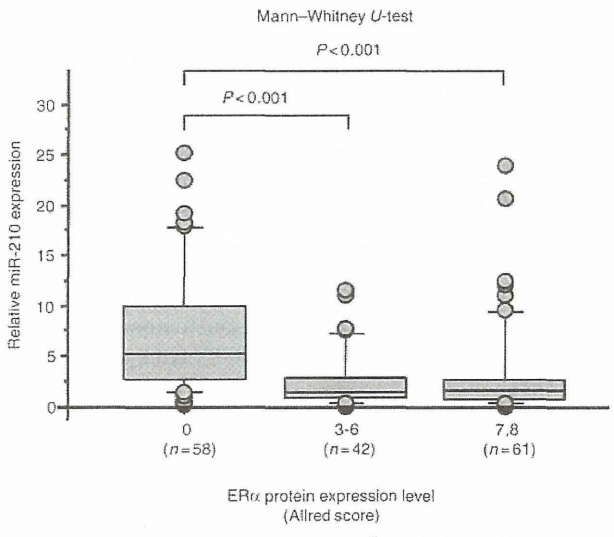

Figure 1. miR-210 expression and estrogen receptor (ERα) protein expression levels according to the Allred score (Allred score 0: $n = 58$; Allred score: 3–6: $n = 42$; Allred score: 7 or 8: $n = 61$). The horizontal line indicates the median concentration, the box covers the 25th–75th percentiles and the maximum length of each whisker shows the 10th or 90th percentiles, respectively. There was no tumor showing ERα protein expression at the level of Allred score 2 in this study.

and high expression (relative miR-210 expression ≥9; $n = 20$; Table 2). The characteristics of patients and their tumors in each group were similar with regard to age, tumor size, nodal status, grade, EGFR protein expression and adjuvant chemotherapy (Table 2). As shown in Fig. 2a and b, patients with TNBCs showing low miR-210 expression had significantly better DFS compared with TNBC patients showing intermediate or high miR-210 expression (log-rank test; $P = 0.03$ and 0.02, respectively), and TNBC patients showing low miR-210 expression also had marginally better OS than did those with high miR-210 expression (log-rank test; $P = 0.05$). The Cox univariate and multivariate analyses demonstrated that miR-210 was an independent prognostic indicator of a poor outcome in TNBCs (Table 3). Next, we studied the relationship between the expression of miR-210 and patient outcome in 40 node-negative TNBCs. Interestingly, the 5-year DFS was ~60% in patients with high miR-210 expression tumors ($n = 11$), while no patient with low-miR-210 expression tumors ($n = 14$) revealed recurrent disease (Fig. 3a and b). DFS and OS curves in intermediate miR-210 expression tumors ($n = 15$) fall between the curves representing low- and high miR-210 expression tumors (Fig. 3a and b).

Table 2. Correlations between miR-210 expression and clinicopathological parameters in TNBCs

	Total ($n = 58$)	miR-210 expression			P value
		Low ($n = 19$)	Intermediate ($n = 19$)	High ($n = 20$)	
Age (years)					
≤50	21	6	7	8	0.77
>50	37	13	12	12	
Tumor size (cm)					
≤2	15	5	4	6	>0.99
>2	43	14	15	14	
Nodal status					
Negative	40	14	15	11	0.22
Positive	17	4	4	9	
Unknown	1	1	0	0	
Grade					
1	3	1	1	1	0.44
2	16	7	5	4	
3	39	11	13	15	
EGFR					
0, 1+	37	14	12	11	0.18
2+, 3+	13	2	5	6	
Unknown	8	3	2	3	
Chemotherapy					
+	42	14	13	15	0.76
−	15	5	6	4	
Unknown	1	0	0	1	

EGFR, epidermal growth factor receptor.

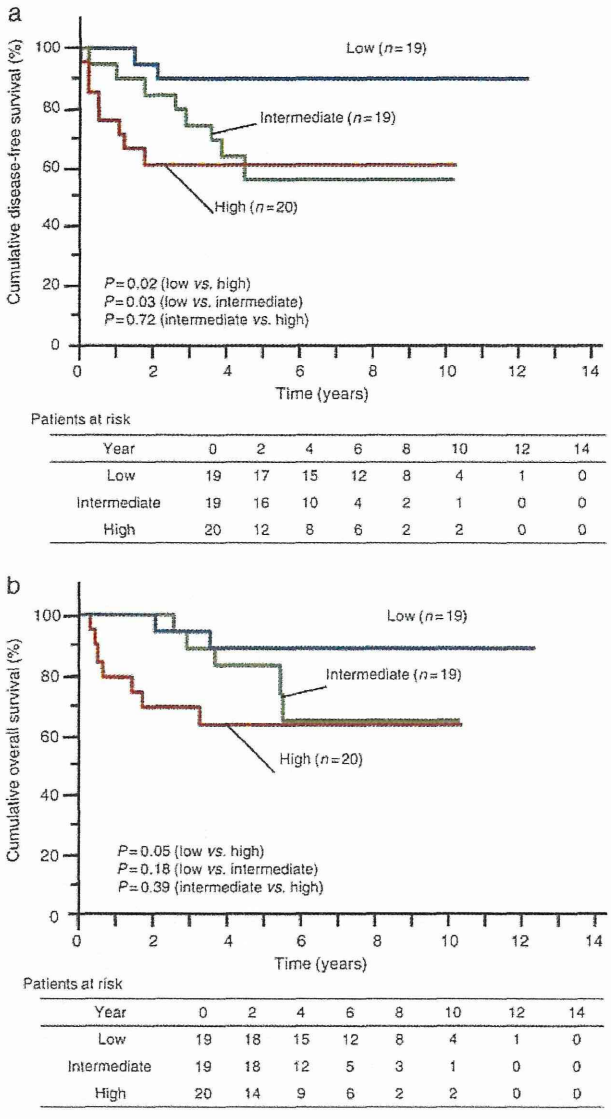

Figure 2. Kaplan–Meier survival curves are shown for disease-free survival (DFS) (a) and overall survival (OS) (b) for all patients with triple negative breast cancer (TNBC; $n = 58$) stratified according to miR-210 expression. The expression levels were stratified by the median value. The DFS (c) and OS (d) are shown for patients with different miR-210 levels. According to miR-210 expression level, TNBCs were divided into three groups.

DISCUSSION

TNBCs are of higher histological grades and have more aggressive clinical behavior than do hormone receptor-positive breast cancers. TNBCs include tumors with the BRCA1 mutation. BRCA1 is a tumor suppressor gene which, when mutated, is associated with the development of hereditary breast cancer. Recently, it has been reported that polyadenosine diphosphate ribose polymerase (PARP) inhibitor, which is believed to induce synthetic lethality in BRCA-deficient cells, might have therapeutic effect in patients with BRCA1 or BRCA2 mutations (25). However, the efficacy of PARP inhibitor in patients without BRCA1 or BRCA2 mutations is unclear. Although effective tailored therapies have been developed for patients with hormone receptor-positive or HER2-positive disease, patients with TNBCs are unlikely to benefit from currently available targeted systemic therapy. Therefore, it is an urgent matter in the treatment of TNBCs that some useful tools are developed and made available, such as new prognostic factors and/or new predictive factors for antitumor agents.

The role of miRNAs in cancer and their potential utility as prognostic factors have become apparent (26). miR-210 has been reported to be highly up-regulated miRNAs in hypoxic cells, and its transcription is regulated by both HIF-1α (7,9,27) and HIF-2α (8). HIF-1α directly binds to an HRE on the proximal miR-210 promoter (7). When the miR-210 core promoter is compared across species, this HRE is highly conserved, indicating the importance of hypoxia in regulating miR-210 expression across species (28). Recently, two groups have reported that a high

Table 3. miR-210 expression in TNBCs. Cox proportional hazards regression analysis

	Univariate	Multivariate	
	P value	*P* value	HR of recurrence (95% CI)
miR-210 expression			
Low			1.0 (reference)
High or intermediate	0.036	0.049	4.39 (1.00 to 19.28)
Nodal status			
Negative			1.0 (reference)
Positive	0.011	0.018	3.06 (1.20 to 7.80)
Tumor size (cm)			
≤2			1.0 (reference)
>2	0.653	0.710	1.23 (0.40 to 3.76)
Grade			
1 or 2			1.0 (reference)
3	0.557	0.280	0.59 (0.22 to 1.53)

HR, hazard ratio; CI, confidence interval.

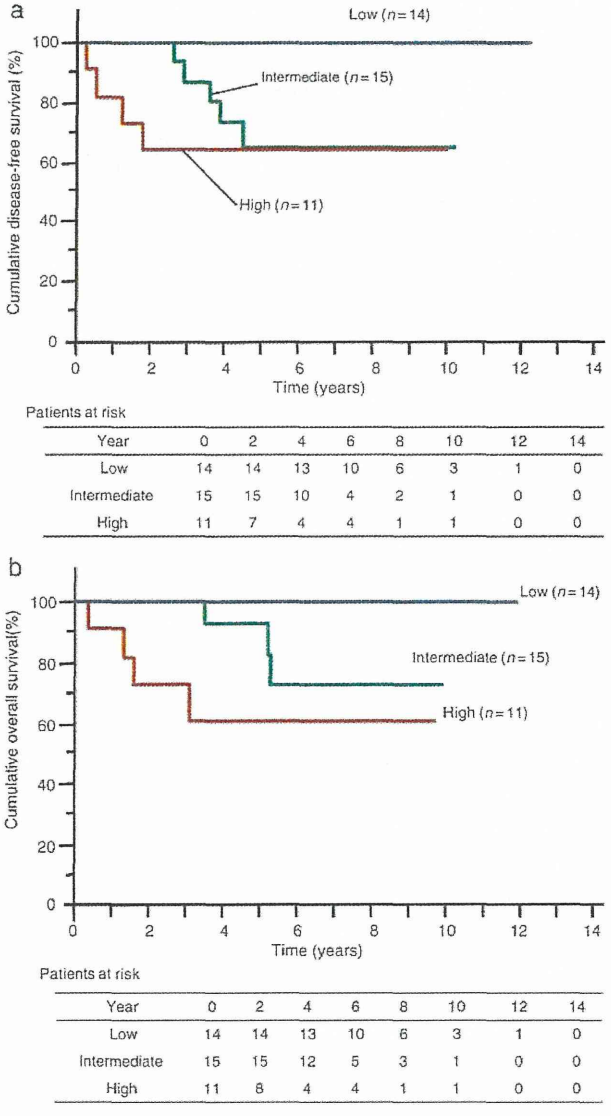

Figure 3. Kaplan–Meier survival curves are shown for DFS (a) and OS (b) for node-negative patients with TNBC ($n = 40$) stratified according to miR-210 expression. According to miR-210 expression level, TNBCs were divided into three groups.

expression of miR-210 in breast cancer patients was associated with a poor prognosis (9,10). As breast cancers are classified into several subtypes and the biology and the clinical outcome are different among its subtypes (17,18,21), we hypothesized that miR-210 expression would vary among them. In this study, we showed that miR-210 expression in TNBCs was significantly higher than that in ER-positive and HER2-negative tumors. Because TNBCs are heterogeneous, we classified the tumors according to their miR-210 expression levels. We found that TNBC patients with low expression of miR-210 had a better prognosis than did those with intermediate- and high miR-210 expression tumors despite the clinicopathological characteristics of the patients, such as tumor grade and the rate of adjuvant chemotherapy, being similar among the three groups. Our data thus supported the report presented by another group (10). Notably, among 40 node-negative TNBCs, the 5-year DFS was ~60% in patients whose tumors had high or intermediate miR-210 expression, while no patient with low miR-210 expression experienced recurrent disease. As we did not perform microdissection to obtain cancer cells, the proportion of the normal cell fraction, e.g. lymphoid cells and stromal cells, in TNBC samples might have influenced our results.

Several targets of miR-210 have been reported recently (7,8,29–31). According to these reports, one of the most important roles of miR-210 appears to be associated with 'mitochondrial dysfunction'. Chan et al. (29) have recently reported that miR-210 targeted the iron–sulfur cluster assembly protein (ISCU), and two other groups also supported this conclusion (30,31). By repressing ISCU1/2 during hypoxia, miR-210 decreases the activity of prototypical iron–sulfur proteins controlling mitochondrial metabolism (29). Consequently, miR-210 represses mitochondrial respiration. Puisségur et al. (11) have also reported that subunit D of succinate dehydrogenase complex (SDHD) is a direct target of miR-210. SDHD is an enzyme of the tricarboxylic acid cycle and a functional member of the mitochondrial respiratory chain (complex II) (32).

The regulation of these mitochondrial components by the miR-210 pathway has contrasting consequences for the regulation of cell death and survival under normoxic or hypoxic conditions (11,31). miR-210 overexpression in normoxia would create a mitochondrial dysfunction including a mismatch in electron transport that could lead to an increase in toxic reactive oxygen species (31) and increased apoptosis

(11,31). miR-210 thus appears to exert a maladaptive role in normoxia (11,31). In contrast, during hypoxia, the miR-210-dependent repression of the electron transport chain via ISCU1/2 and SDHD would be protective by participating in the homeostatic down-regulation of mitochondrial respiration (6,11,29,31).

The reduced mitochondrial respiration could be responsible for the tumor cell growth advantage in a hypoxic microenvironment. One hypothesis suggests that a reduced mitochondrial function could conserve O_2 for alternative use (33). When the distance travelled by O_2 to reach the outermost cell was defined as the diffusion limit, the limit is a function of the O_2 content within the blood vessels and the rate of consumption within the tumor cells as O_2 diffuses out from the vessels. Decreasing consumption is theoretically the most efficient way to extend this diffusion limit *in vivo* (33,34). The second hypothesis suggests that a reduced mitochondrial function could yield an increase in anabolic substrates (33). Rapidly dividing tumor cells require large amounts of precursors for proteins, nucleic acids and lipids (35). An increased glucose uptake by tumor cells and a decreased consumption of metabolites for energy in the mitochondria provide more substrates for lipid and nucleic acid synthesis (33).

Tumors with high miR-210 expression might have a growth advantage via reduced mitochondrial respiration in a hypoxic microenvironment. Because TNBCs usually have aggressive clinical behavior, they could have some areas of hypoxic microenvironment inside their tumors, where miR-210 could be highly expressed. On the other hand, HR-positive tumors are usually of lower histological grade and less aggressive than TNBCs. We demonstrated that miR-210 expression in TNBCs was higher than in HR-positive tumors. We consider that our data support the hypothesis described above. However, Camps et al. (9) have reported no interaction between ER and miR-210 expression levels. They evaluated ER status by ELISA while ER expression was confirmed by IHC analysis in the present study. Therefore, the methodology for evaluation of ER status might be responsible for these different results.

In this study, patients with TNBCs that expressed low levels of miR-210 had a more favorable prognosis, although the prognosis of TNBCs is relatively poor. Thus, the degree of miR-210 expression might be a clinically useful prognostic factor for decision-making regarding treatment in the adjuvant setting, especially in node-negative TNBC patients. Although we used frozen samples for this study, miRNA assays could also have been performed using formalin-fixed paraffin-embedded samples because technology has been rapidly advancing in this field. Although the multifaceted roles of miR-210 have been gradually clarified under hypoxia and normoxia status, the biological functions of miR-210 that are involved in providing a growth advantage for TNBCs are not fully understood. Further research might help to reveal that miR-210 could be not only a prognostic factor, but also a therapeutic target in TNBCs.

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Conflict of interest statement

None declared.

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Perceived Needs, Psychological Distress and Quality of Life of Elderly Cancer Patients

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Objective: Very few findings regarding the perceived needs of elderly cancer patients have been reported. This study investigated needs and psychological distress perceived by and/or quality of life of elderly cancer patients.
Methods: Randomly selected ambulatory patients with cancer participated in this study. The patients were asked to complete the Short-form Supportive Care Needs Survey questionnaire, which covers five domains of need (health system and information, psychological, physical, care and support, and sexual); the Hospital Anxiety and Depression Scale and the European Organization for Research and Treatment of Cancer QLQ-C 30.
Results: Complete data were available for 619 cancer patients, including 113 subjects who were over 70 years old. The needs and the psychological distress perceived by the elderly patients were comparable with those perceived by relatively younger patients, although elderly patients perceived fewer sexual needs. Regarding the quality of life global health status, most symptom-related quality of life parameters were not significantly different between the two groups, while significant differences were observed with regard to several functional domains, including physical, emotional and social domains in addition to financial difficulties.
Conclusions: Only a few differences in the needs and the psychological distress perceived by patients existed between the elderly and the younger subjects, although some differences in the quality of life domains were noted, probably as a result of the influence of aging itself. Medical staff should provide elderly cancer patients with good clinical care similar to that provided to younger patients while considering the different impacts of aging on each quality of life dimension.

Key words: oncology – elderly need – psychological distress – quality of life – supportive care

INTRODUCTION

One of the most important risk factors for the development of cancer is aging because of which more than half of all new cancers occur in elderly people (1). In Japan, which currently has the greatest life expectancy at birth in the world (averaging 83 years for men and women), cancer has continuously been the leading cause of death since 1981, and more than one-third of Japanese people die of cancer (2). Thus, Japan has attracted the attention of many countries as one of the most rapidly aging societies in the world, and Japan will become a super-aging society with people aged 65 years and over accounting for 25% of the population in the near future.

Needless to say, providing appropriate patient-centered care as well as optimal active anti-cancer treatment to elderly cancer patients is part of good clinical oncology practice. Nevertheless, very few studies have specifically focused on the treatment and care for elderly cancer patients, as many studies exclude elderly patients (3). Furthermore, since elderly cancer patients are physically, psychologically and socially heterogeneous in addition to differing from younger patients with regard to their physical functioning, psychological well-being, life circumstances, role demands, values and preferences, the treatment and care for elderly cancer patients is complex, especially considering individualized optimal care (1,4,5).

Several previous studies have investigated differences in quality of life (QOL) between young and old cancer patients, and many of these studies have revealed that older subjects' emotional, social and economical functioning are generally better and their physical functioning is worse than that of their younger counterparts, despite the absence of significant differences in their total and/or global QOL (6–9). Concerning other relevant outcomes, including patients' psychological distress and supportive care needs, older cancer patients experience similar or somewhat less psychological distress and generally have less information and sexual needs (10–12,13), although very few studies have investigated age differences among other domains of patients' supportive care needs.

Findings regarding older cancer patients' supportive care needs are essential because the assessment of needs offers a number of advantages. First, needs perceived by patients and patient-important outcomes can be directly assessed, enabling a more direct determination of necessary resources. Actually, the patients' problems and symptoms do not necessarily reflect the actual needs (14). Second, such assessments enable the magnitude of a particular need to be identified, thereby allowing some prioritization of service needs so that the available resources can be allocated where the need is most urgent. Third, an assessment of needs enables identification of individuals and/or patient subgroups with higher need levels, thereby enabling prevention or minimization of problems through appropriate early interventions (15). Thus, understanding the needs perceived by elderly patients will enable medical staff to develop services or procedures specially designed to meet them, providing valuable guidance for forthcoming super-aging societies.

To the best our knowledge, no large study has investigated age-specific differences regarding QOL and psychological distress in addition to supportive care needs among elderly cancer patients in an Asian country.

The purposes of this study were: (i) to investigate age-specific differences in supportive care needs in addition to psychological distress and QOL of elderly cancer patients and (ii) to determine the frequency of unmet supportive care needs in such individuals. Our hypotheses were that elderly cancer patients generally perceive fewer supportive care needs and less psychological distress, compared with younger patients, because our previous studies demonstrated that older cancer patients are less likely to be referred to the psycho-oncology service and that the incidence of psychiatric morbidity is lower (16,17).

PATIENTS AND METHODS

Participants and Procedures

This study was conducted with data obtained from our two previous studies that were published in 2010 and 2011. The first study subjects were randomly sampled ambulatory cancer patients of the outpatient oncology unit at Nagoya City University Hospital (Study 1) (18). The other study subjects were randomly sampled ambulatory female patients with breast cancer attending the outpatient clinic for Oncology, Immunology and Surgery at Nagoya City University Hospital (Study 2) (19).

The eligibility criteria for inclusion in both the studies were as follows: (i) a diagnosis of cancer (all stages and any time point after diagnosis), (ii) an age of 20 years or older, (iii) an awareness of the diagnosis of cancer and (iv) a general condition sufficient to enable the completion of the survey questionnaire [0-3 on the Eastern Cooperative Oncology Group (ECOG) performance status]. The exclusion criteria were patients with (i) severe mental or cognitive disorders (e.g. uncontrolled schizophrenia, dementia and delirium) or (ii) an inability to understand the Japanese language.

Both the studies were approved by the Institutional Review Board and Ethics Committee of Nagoya City University Graduate School of Medical Sciences, Japan, and were conducted in accordance with the principles outlined in the Helsinki Declaration. Written consent was obtained from each patient after a thorough explanation of the purpose and the methods involved in the study had been provided.

Procedure

After informed consent had been obtained, the patients were asked to complete the following self-administered questionnaires (described in the following section) at home and to

return them the following day. If any of the questions were answered inadequately, clarifications were sought over the telephone.

Patients' Perceived Needs: The Short-form Supportive Care Needs Survey Questionnaire

The Short-form Supportive Care Needs Survey (SCNS-SF34) is a self-administered instrument for assessing the needs perceived by patients with cancer (20,21). The SCNS-SF34 consisted of 34 items covering five domains of need: psychological (10 items), health system and information (11 items), physical and daily living (5 items), patient care and support (5 items) and sexual (3 items). The respondents were asked to indicate the level of their need for help over the previous month in relation to their cancer, using the following five response options: [1, No Need (Not applicable); 2, No Need (Satisfied); 3, Low Need; 4, Moderate Need and 5, High Need]. Subscale scores were obtained by summing the individual items. In addition, the total score was obtained by summing all the subscales (range, 34–170). A higher score indicated a higher perceived need. Besides, the scale could be used to obtain information on the presence/absence and the number of unmet perceived needs (a rating of three or higher was regarded as an unmet need), depending on the researcher's question on a clinical aspect. The validity and the reliability of the Japanese version of the SCNS-SF34 have been established (22).

Psychological Distress: Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) has been developed for use in medically ill patients and does not contain any questions regarding physical symptoms (23). The HADS is a self-reported questionnaire consisting of 14 items. The subjects are asked to rate on a 4-point Likert scale how they felt during the previous week. The HADS consists of an anxiety and a depression subscale (0–21 points each), and the total score can range from 0 to 42. A higher score indicates more severe depression and anxiety. The Japanese version of the HADS has been validated for cancer populations (24). The optimal cut-off point for screening for adjustment disorders and/or major depressive disorders (indicating psychological distress) was 10/11, while the cut-off for major depression (indicating serious psychological distress) was 19/20.

Quality of Life

The QOL of the patients was assessed using the European Organization for the Research and Treatment of Cancer (EORTC) QLQ-C30 (25). The QLQ-C30 is a 30 item, self-reported questionnaire covering functional (Global Health Status, Physical Functioning, Role Functioning, Emotional Functioning, Cognitive Functioning and Social Functioning) and symptom-related aspects (fatigue, nausea and vomiting, pain, dyspnea, insomnia, appetite loss, constipation, diarrhea and financial difficulties) of QOL in cancer patients. The validity and the reliability of the Japanese version of the EORTC QLQ-C30 have been confirmed (26). A high functional score represents a high QOL. A high symptom score indicates a strong symptom.

Sociodemographic and Biomedical Factors

An *ad hoc* self-administered questionnaire was used to obtain information on the patients' sociodemographic statuses, including their marital status, level of education and employment status. The performance status, as defined by the ECOG, was evaluated by the attending physicians and was an objective index of a patient's physical functioning, ranging from 0 (no symptoms) to 4 (bedridden). All other medical information (duration since diagnosis, clinical stage and anti-cancer treatment) was obtained from the patients' medical records.

Statistical Analysis

To investigate the differences regarding the needs and psychological distress perceived by and/or QOL of the patients between two different age groups, an unpaired *t*-test and a Mann–Whitney test were conducted, as appropriate. We chose 70 years of age as the cut-off in this study because many age-related changes occur after this age (27). After these univariate analyses, we used an analysis of covariance (ANCOVA) to examine whether the differences were observed even after adjustments for potential confounding factors, including sex, cancer site (breast cancer vs. other cancers), clinical stage (IV or recurrence vs. other stages) and performance status (defined by ECOG).

To determine the frequencies of perceived needs of the elderly patients, data only for the subjects in their 70s were extracted. Then, each score for the 34 items of need was used (a rating of three or higher was regarded as the presence of an unmet need) to evaluate the frequencies of individual unmet perceived needs.

A P value of < 0.05 was regarded as statistically significant, and all the reported P values were two-tailed. All statistical procedures were conducted using the SPSS 18.0J version software for Windows (SPSS Inc., 2009).

RESULTS

Patient Characteristics

A total of 619 patients (response rate, 93%), including 405 subjects from Study 1 (cancer patients attending the outpatient oncology unit) and 214 subjects from Study 2 (breast cancer patients attending the outpatient clinic), participated in the study. The sociodemographic and clinical characteristics of these 619 patients are shown in Table 1. Among

Table 1. Participant characteristics

Characteristics	All	%	≥70 years	%	<70 years	%
No.	619	100	113	18	506	82
Sex						
Female	536	87	86	76	450	89
Male	83	13	27	24	56	11
Marital status						
Married	467	75	68	60	399	79
Living with others						
Present	549	89	92	81	457	90
Education						
>12 years	227	37	18	16	209	41
Employment status						
Full-time/part-time	248	40	20	18	228	45
Cancer site						
Breast	480	78	65	58	415	82
Colorectal	55	9	18	16	37	7
Lung	19	3	8	7	11	2
Lymphoma	16	3	3	3	13	3
Stomach	14	2	7	6	7	1
Others	35	6	12	11	23	5
Clinical stage						
Recurrent/metastatic	193	31	53	47	140	28
Performance status[a]						
0	536	87	90	80	446	88
1	72	12	17	15	55	11
2	8	1	4	4	4	1
3	3	1	2	2	1	0.2
Current anti-cancer treatment[b]						
Chemotherapy	265	43	57	50	208	41
Radiation therapy	14	2	0	0	14	3

[a]Eastern Cooperative Oncology Group criteria.
[b]Multiple choice.

Table 2. Differences in needs, psychological distress and quality of life (QOL) between the elderly and the younger subjects

Variables	Mean (SD)		*P*	*P*[a]
	≥70 years	<70 years		
Needs				
Psychological	24.0 (10.3)	24.2 (10.5)	0.87	—
Health system and information	29.6 (11.5)	27.0 (11.8)	0.04	0.11
Physical and daily living	9.8 (4.3)	9.7 (4.4)	0.90	—
Patient care and support	11.2 (4.6)	10.6 (4.6)	0.21	—
Sexual	3.9 (2.0)	4.6 (2.4)	<0.01	<0.01
Total score	78.5 (26.7)	76.1 (28.3)	0.40	—
Psychological distress				
Anxiety	4.5 (3.2)	4.9 (3.7)	0.26	—
Depression	5.1 (3.5)	4.9 (3.7)	0.53	—
Total score	9.6 (6.0)	9.8 (6.9)	0.81	—
Quality of life				
Global Health Status	65.3 (20.8)	62.7 (23.4)	0.28	—
Physical functioning	78.5 (20.1)	85.8 (15.5)	<0.01	0.02
Role functioning	78.0 (26.4)	76.4 (26.1)	0.56	—
Emotional functioning	83.6 (16.6)	78.5 (19.8)	0.01	<0.01
Cognitive functioning	75.4 (18.4)	79.2 (19.7)	0.06	—
Social functioning	84.5 (20.1)	79.7 (23.9)	0.03	<0.01
Fatigue	34.6 (22.5)	32.9 (22.9)	0.48	—
Nausea and vomiting	5.6 (12.3)	8.6 (18.1)	0.03	<0.01
Pain	16.8 (22.0)	20.2 (21.3)	0.13	—
Dyspnea	19.2 (22.6)	15.9 (21.7)	0.16	—
Insomnia	19.8 (24.7)	22.3 (25.1)	0.34	—
Appetite loss	18.3 (26.0)	15.9 (25.3)	0.36	—
Constipation	21.5 (25.9)	20.6 (25.1)	0.73	—
Diarrhea	11.5 (21.7)	9.9 (19.5)	0.45	—
Financial difficulties	13.9 (22.1)	27.4 (31.7)	<0.01	<0.01

[a]Adjusted for sex, cancer site, cancer stage and performance status if a significant difference was observed using a univariate analysis.

them, 113 subjects (18%) were more than 70 years old, of whom approximately two-thirds and one-half were married and had recurrent/metastatic cancer, respectively. The most common cancer site was the breast in both groups. The background characteristics of the two age-specific subject groups were similar, as shown in Table 1.

Age-specific Differences in Supportive Care Needs, Psychological Distress and QOL

Findings with regard to age-specific differences in supportive care needs, psychological distress and QOL are shown in Table 2. Regarding supportive care needs, the elderly subject group showed fewer sexual needs, but no significant age-specific differences were observed among the other need domains. Concerning psychological distress, both the age groups experienced similar incidences of anxiety and depression. Although the global health status was similar in the two age groups, several significant age-specific differences in the QOL domains were observed. The elderly subjects had a lower physical functioning but they had better emotional functioning, better social functioning, less nausea/vomiting and fewer financial difficulties.

Frequency of Unmet Needs of the Elderly

The most common unmet need (rated three or more on the 5-point Likert scale) is shown in Table 3. 'Fears of spreading

Table 3. Prevalence of the 10 most common unmet needs[a] among the elderly (≥70 years) cancer patients

Unmet needs	Needs domain	%
1. Fears of spreading of cancer	Psychological	66
2. Concerns about the worries of those close to you	Psychological	58
3. Worry that the results of treatment are beyond your control	Psychological	58
4. Being informed about things you can do to help yourself get well	Health system and information	53
5. Being informed about cancer which is under control or diminishing	Health system and information	50
6. Being adequately informed about the benefits and side-effects of treatments before you choose to have them	Health system and information	49
7. Being informed about your test results as soon as feasible	Health system and information	49
8. Anxiety	Psychological	48
9. Being given explanations of those tests for which you would like explanations	Health system and information	47
10. Being given information (written, diagrams, drawings) about aspects of managing your illness and side-effects at home	Health system and information	47

[a]Rated three or more on a 5-point Likert scale for each item of the Short-form Supportive Care Needs Survey questionnaire.

of cancer' was the most common, followed by 'Concerns about the worries of those close to you', 'Worry that the results of treatment are beyond your control', 'Being informed about things you can do to help yourself to get well' and 'Being informed about cancer, which is under control or diminishing'. The prevalence of the five most common unmet needs was over 50%, and all of these unmet needs belonged to the psychological domain or the health system and information domain. Each patient had a mean ± SD of unmet needs of 13 ± 10. The mean ± SD values of the unmet needs in each domain were as follows: psychological needs (10 items), 4.8 ± 4.0; health system and information (11 items), 4.9 ± 4.4; physical and daily living needs (5 items), 1.7 ± 1.9; patient care and support needs (5 items), 1.2 ± 1.9 and sexual needs (3 items), 0.2 ± 0.7.

DISCUSSION

The needs as well as the psychological distress perceived by the elderly patients were almost comparable with those perceived by the relatively younger cancer patients, although the elderly patients perceived fewer sexual needs. Furthermore, the global health status and most symptom-related QOL parameters were not significantly different between the two groups, while significant differences in several functioning scores, including physical, emotional and social ones, in addition to financial difficulties were observed. In contradiction to our hypotheses, the elderly cancer patients generally perceived similar supportive care needs and levels of psychological distress, compared with the relatively younger patients.

With regard to differences in perceived supportive care needs, those among the elderly subjects were similar to those among the younger subjects, with the exception of sexual needs. While the observed difference in sexual needs is not unexpected, several other findings are interesting. In particular, the similar needs for health system and information among the older and the younger subjects was somewhat unexpected, since many previous studies have demonstrated that information needs among elderly patients are generally fewer than among younger subjects (12,13). On the other hand, while many studies have specifically focused on information needs regarding so-called 'bad news', such as cancer diagnosis and prognosis, the needs questionnaire used in the current study concerned needs regarding good news ('Being informed about cancer which is under control or diminishing [that is, remission]'), not bad news. Although the general cancer-related information needs were similar for the two age groups, the elderly patients were not more likely to need information regarding 'bad news' (10). These findings suggest that medical staff members should carefully consider the quality and quantity of communication with elderly patients. Furthermore, elderly patients may generally perceive a similar degree of supportive care needs, compared with relatively younger patients, with the exception of sexual needs and 'bad news'.

This study demonstrated that the amount of information and the number of psychological needs were relatively high among elderly cancer patients, compared with the other need domains, and this finding was consistent with the results of previous studies conducted in cancer patients (28–31). In particular, regarding the individual unmet needs among elderly patients, 'Fears of spreading of cancer' was the most common, and approximately two-thirds of the subjects perceived a need for help. One Japanese survey that investigated the concerns of 7885 cancer patients reported that the most common concerns were over problems associated with the psychological domain, of which fears of recurrence of cancer and/or metastasis was the most common problem (32). These findings as well as previous findings suggest that medical resources and/or the development of an interventional program for the reduction of fears/anxieties associated

with recurrence and spreading of cancer are needed for the care of cancer patients, (33) as very few strategies exist for the management of specific sources of distress (34). Since cancer patients' 'Fears of spreading of cancer ' are generally differentiated from anxiety disorders, which are characterized by irrational fears/anxieties, in psychiatric nosology, the development of a novel intervention program is needed to address these needs (35).

With regard to psychological distress, no significant differences were observed between the elderly and the younger patients. Although some previous studies found that relatively younger cancer patients were more likely to experience severe psychological distress, (16) this study and a recent meta-analysis have shown that age may not be a strong contributing factor to patients' psychological distress (36). As described later, despite the absence of a difference in psychological distress, the finding that elderly patients can maintain better emotional functioning suggests a relatively smaller impact of psychological distress on daily life. Nevertheless, the current findings suggest the need for appropriate support for elderly patients with psychological distress.

There were several interesting findings regarding age-specific differences in the QOL domains. The elderly patients had a lower physical functioning, suggesting that the influence of aging on the physical status may be reflected in the QOL results. On the other hand, concerning the symptom-related QOL, the elderly patients had less nausea/vomiting. While the reason for this finding is uncertain, elderly patients may be unlikely to receive highly cytotoxic chemotherapeutic agents. Furthermore, the elderly patients showed better social functioning and experienced fewer financial difficulties. Differences in life stages, such as the fact that elderly patients have completed their parenting responsibilities and have retired from work, may explain these results. These results suggest that elderly patients may have unique QOL issues in some domains, and the assessment of the impact of aging on the individual QOL may be essential for a better care for elderly cancer patients. Thus, a comprehensive geriatric assessment may be desirable for elderly patients.

This study had several limitations. First, the data on comparison with healthy elderly subjects required for a rigorous investigation into the influence of aging on cancer patients' psychosocial outcome are unfortunately not available. Second, as this was a cross-sectional study, we could not rule out the possible unreliability of the findings. Third, since supportive care needs can be influenced by the patients' cultural backgrounds and the medical system of the country they belong to, the findings may not be applicable to other patient populations. Fourth, since the study was conducted at one institution, an institutional bias may exist. Last, since this study focused on ambulatory cancer patients and relatively few patients with low physical functioning were enrolled, the results may not be applicable to inpatients and patients with terminal cancer. Especially, low physical functioning of elderly patients leads to more needs, further studies are needed to address the needs of elderly patients with low physical functioning.

In conclusion, only a few differences in the needs and psychological distress perceived by patients existed between the elderly and the younger subjects, although some differences in the QOL domains may have arisen mainly as a result of the aging process itself. Medical staff should provide elderly cancer patients with good clinical care similar to that provided to younger patients, considering the impact of aging on each dimension of the QOL.

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Conflict of interest statement

None declared.

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Randomized phase II study of three doses of oral TAS-108 in postmenopausal patients with metastatic breast cancer

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This randomized phase II study was intended to identify the optimal dose of TAS-108, a novel steroidal antiestrogen, for the treatment of breast cancer in postmenopausal Japanese women. The potential clinical effects of TAS-108 on the uterus, bone, serum lipids, and hormones were also investigated. Postmenopausal women with hormone receptor-positive metastatic breast cancer who had previously received one or two endocrine therapies were randomly assigned to one of the three possible dose levels of TAS-108 (40, 80 or 120 mg/day). Oral TAS-108 was given daily, and the efficacy and safety of the three doses were evaluated. A total of 97 patients (33, 32, and 32 in the 40-, 80-, and 120-mg groups, respectively) were treated with TAS-108. The clinical benefit rate was 30.3% for the 40-mg, 25.0% for the 80-mg, and 25.0% for the 120-mg group. The 40-mg group achieved the prespecified target threshold. TAS-108 at all dose levels was well tolerated and appeared to have no harmful effects in terms of the variables examined in this study. We conclude that the optimal dose of TAS-108 among the three doses is 40 mg, once daily, for further studies. JAPIC Clinical Trials Information number: Japic CTI – 121754. (*Cancer Sci* 2012; 103: 1708–1713)

Aromatase inhibitors have been widely used as first-line endocrine therapeutic agents for postmenopausal patients with HR-positive breast cancer and also adjuvant therapy in postmenopausal women with early breast cancer.[1,2] However, tamoxifen showed equivalent disease-free survival compared with AIs in patients with low tumor values of Ki-67 in an adjuvant trial,[3] and it has also been reported that tamoxifen holds potential for sequential treatment of postmenopausal patients with MBC progressing after AI treatment.[4] Therefore, tamoxifen still remains an important treatment option in HR-positive breast cancer. However, due to its estrogen-like effects on the uterus, tamoxifen has been associated with the risk of developing endometrial cancer,[5] which has been an important motivating factor in the development of new types of antiestrogen with different pharmacologic profiles.

A novel steroidal antiestrogenic compound, TAS-108 binds strongly to ERα and ERβ with a mechanism of action unlike tamoxifen,[6] and in humans is mainly metabolized by CYP3A4 enzymes in the liver.[7] TAS-108 shows pure antagonistic activity as it blocked both the N-terminal AF-1 and C-terminal AF-2 transactivation functions of ERα, abolished the recruitment of co-activators, but promoted the recruitment of co-repressors and allowed normal DNA binding. Additionally, TAS-108 has shown antagonistic effects on a mutant ERα reported to have a tamoxifen-resistant phenotype and preliminarily shown to have antitumor activity against tamoxifen- and AI-resistant cell lines.[8] TAS-108 has also shown fewer estrogenic effects on the uterus than tamoxifen in animal models.[6] Furthermore, a preclinical study suggested possible positive effects of TAS-108 on BMD.[9]

Several phase I studies were carried out in the USA involving postmenopausal healthy women and MBC patients.[10,11] In these studies, TAS-108 was well tolerated at all doses of 40–160 mg, and showed possible antitumor activity.

Two phase I studies of TAS-108 in Japan in 12 postmenopausal healthy women[12] and 15 MBC patients,[13] involving doses of 40, 80, or 120 mg showed a favorable safety profile, and encouraging antitumor activity in the MBC group. However, these studies were not designed to establish the optimal dose of TAS-108.

The present multicenter study was carried out to evaluate both the efficacy and safety of three different TAS-108 doses, and subsequently to identify the optimal dose of TAS-108 for further studies in postmenopausal Japanese patients with MBC. Considering long-term use, especially in the adjuvant setting, the effect on aspects not directly related to cancer would be especially important for administration in postmenopausal women. We also investigated the potential clinical impact of TAS-108 on the uterus, bone, serum lipids, and hormones.

Patients and Methods

Study design and treatment. In this multicenter, randomized, non-blinded phase II study carried out in Japan, patients were randomly assigned to receive oral TAS-108 with a daily dose

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of either 40, 80 or 120 mg (in units of 40 mg tablets; Taiho Pharmaceutical, Tokyo, Japan) after the first daily meal for 24 weeks or until disease progression, development of unacceptable toxicity, or withdrawal of consent. Patients with favorable response (CR, PR, or SD) at 24 weeks could continue the treatment. Two stratification factors: response to prior endocrine treatment, and presence of visceral metastasis, were used to balance the patient populations among the three dose groups at randomization.

At baseline, a full medical history was taken and a physical examination carried out. Patients also underwent clinical laboratory tests, examination of vital signs, electrocardiogram, and PS evaluation. At 2 and 4 weeks after initiating drug intake, and subsequently every 4 weeks, evaluations were carried out, including physical examination, toxicity assessment, and clinical laboratory tests. Endometrial thickness was measured by transvaginal (transabdominal) ultrasonography at baseline and every 24 weeks during treatment. Lumbar spine (L2–L4) BMD was assessed by dual energy X-ray absorptiometry at baseline and after 24 weeks of treatment. Serum hormones (E2, FSH, prolactin, thyroid-stimulating hormone, cortisol, testosterone, and sex hormone-binding globulin), serum lipid (apolipoprotein A-I and B) and BMMs (serum osteocalcin and I-CTP) were assessed at baseline and at regular intervals (measured at SRL Medisearch, Tokyo, Japan). All blood tests including for serum lipids (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides), were carried out on specimens obtained before the first daily meal.

Eligibility criteria. Postmenopausal HR-positive women aged 20–80 years with histologically or cytologically proven, locally advanced or MBC, were eligible for the study if they appeared suitable for endocrine therapy. The postmenopausal status was defined as being amenorrheic for at least 1 year (for patients aged 50 years or over), being amenorrheic for at least 1 year and with both serum E2 and FSH levels in the postmenopausal range, or being amenorrheic due to radiotherapy for at least 3 months with both E2 and FSH levels in the postmenopausal range (for patients aged under 50 years). All patients had to have at least one progressive target lesion after one or two different endocrine therapies. Patients could have received one prior chemotherapy regimen, unless it had been given as the most recent prior treatment. Patients who had had only adjuvant endocrine therapy were eligible if they had relapsed during therapy or <6 months from the completion or discontinuation of the therapy. Other inclusion criteria included: adequate organ function; a predicted life expectancy of >3 months; PS of 2 or less on the Zubrod scale.

Patients were ineligible if they had allergies to steroid preparations; abnormal vaginal bleeding at the start of the treatment; past serious thromboembolism; current serious complication(s); active double cancer; inflammatory breast cancer, lung metastasis with cancer-related lymphangitis, brain metastasis with any symptoms, and widespread liver metastasis.

The study was approved by the institutional review board of each participating center. Written informed consent was obtained from all patients.

Efficacy and safety assessments. Tumor response assessments were carried out at baseline and at 8-week intervals. The response was assessed according to the Response Evaluation Criteria in Solid Tumors criteria.[14] For CR and PR, the response had to be confirmed more than 4 weeks after the first date when a response was documented. The efficacy results were reviewed and determined by the independent CEC. Retrospective analyses for the response to TAS-108 were carried out to explore the subgroup, including patients who had tamoxifen- or AI-resistant tumors, defined as patients who had: (i) previously failed to respond to the most recent prior treatment for advanced disease; (ii) progressed following response to treatment; and (iii) relapsed either on adjuvant therapy or within 6 months from the completion of adjuvant therapy. Adverse events were graded according to the Common Terminology Criteria for Adverse Events, version 3.0.

Statistical considerations. The primary end-point was the CBR at 24 weeks, defined as the percentage of eligible patients who achieved a CR, PR, or SD for at least 24 consecutive weeks. The secondary end-points included ORR (CR or PR) and TTP. The secondary end-points also included safety and effects on ET, BMD, BMMs, serum lipids, and hormone levels.

We considered 35% as a clinically meaningful CBR and that that would be the expected CBR of TAS-108 in the study population, whereas a 10% CBR would be considered poor and lacking promise for future development. Sample size was estimated to ensure both appropriate precision for CBR estimation in all evaluable patients and sufficient statistical power to reject the null hypothesis with adjusted significance level for multiple comparisons. At least a total of 84 evaluable patients, 28 in each dose group, were required to carry out binomial tests for $P_1 = 35\%$ and $P_0 = 10\%$ in each dose group with 2.5% family-wise one-sided type-I error level with 80% statistical power in each test. For CBR estimation in total, and assuming a 5% drop-out rate, a total of 96 patients, 32 in each dose group, were planned to be enrolled in the study.

The Kaplan–Meier method was used to estimate TTP, which was defined as the time from first drug administration to disease progression. The 98.3% exact binomial CI was estimated for CBR and ORR in each dose group.

In order to explore the potential clinical impact of TAS-108 on the uterus, bone, serum lipids, and hormones, we carried out non-parametric analysis. Bone mineral density and BMMs were assessed in patients with no bone metastasis at baseline. Because of uncertainty regarding the asymptotic normality of changes in variables, the Wilcoxon signed-rank test was used to assess the significance of changes from baseline within each dose group with a value <0.05 considered as statistically significant.

Results

Patient population. A total of 98 patients were enrolled at 34 centers in Japan (Appendix I). One patient randomized to the 120-mg group was censored and did not receive any TAS-108 treatment due to not having had endocrine therapy as the most recent prior treatment. The treated patient population therefore comprised 97 patients who were fully assessable for efficacy and safety; 33 in the 40-mg group, and 32 each in the 80-mg and 120-mg groups. The baseline characteristics of the patients were well balanced among the three dose groups (Table 1). The study population included 14 patients with metastatic disease refractory to prior tamoxifen treatment and 70 were refractory to prior AI treatment.

Efficacy. The CBR determined by CEC was 30.3% (98.3% CI, 13.3–52.4) in the 40-mg group, 25.0% (98.3% CI, 9.5–47.2) in the 80-mg group, and 25.0% (98.3% CI, 9.5–47.2) in the 120-mg group, respectively (Table 2). The 40-mg group exceeded the target threshold of 10% in its lower limit of CI. The CEC-determined ORR was 9.1% (98.3% CI, 1.3–27.9) in the 40-mg group, 9.4% (98.3% CI, 1.3–28.6) in the 80-mg group, and 6.3% (98.3% CI, 0.4–24.3) in the 120-mg group, respectively (Table 2). The median TTP was 4.6 months in the 40-mg group, 3.7 months in the 80-mg group, and 3.6 months in the 120-mg group (Table 2). The Kaplan–Meier curve of TTP is shown in Figure 1.

In the subgroup analysis of the patient population of tumor refractory to tamoxifen or AI, TAS-108 treatment produced a CBR of 28.6% and 27.1%, respectively (Table 3).

Table 1. Patient characteristics at baseline

Characteristics	Dose group			Total
	40 mg (*n* = 33)	80 mg (*n* = 32)	120 mg (*n* = 32)	(*n* = 97)
Median age, years (range)	63.0 (44–80)	63.0 (50–74)	58.5 (48–78)	62.0 (44–80)
Performance status, *n* (%)				
0	28 (85)	27 (84)	28 (88)	83 (86)
1	5 (15)	5 (16)	4 (13)	14 (14)
2	0 (0)	0 (0)	0 (0)	0 (0)
Body mass index (median), kg/m^2	23.2	23.0	22.1	22.7
Prior treatment, *n* (%)†				
Endocrine therapy regimens				
1	19 (58)	13 (41)	21 (66)	53 (55)
2	14 (42)	19 (59)	11 (34)	44 (45)
Chemotherapy regimens				
0	21 (64)	28 (88)	23 (72)	72 (74)
1	12 (36)	4 (13)	9 (28)	25 (26)
Sites of metastasis, *n* (%)				
Soft tissue	20 (61)	23 (72)	19 (59)	62 (64)
Bone	12 (36)	15 (47)	17 (53)	44 (45)
Visceral	23 (70)	23 (72)	23 (72)	69 (71)
Other	2 (6)	0 (0)	4 (13)	6 (6)
Receptor status, *n* (%)‡				
ER+/PgR+	22 (67)	20 (63)	19 (59)	61 (63)
ER+/PgR−	11 (33)	9 (28)	11 (34)	31 (32)
ER−/PgR+	0 (0)	2 (6)	1 (3)	3 (3)
ER−/PgR−	0 (0)	0 (0)	0 (0)	0 (0)
HER2+	4 (12)	4 (13)	7 (22)	15 (15)
HER2−	24 (73)	23 (72)	23 (72)	70 (72)
HER2 unknown	5 (15)	5 (16)	2 (6)	12 (12)
Disease-free interval, *n* (%)				
<2 years	5 (15)	5 (16)	5 (16)	15 (15)
≥2 years	21 (64)	22 (69)	17 (53)	60 (62)

†Counting a case treated with adjuvant endocrine therapy/chemotherapy as one regimen, if it had relapsed either during therapy or within 6 months of completion of therapy. ‡Hormone receptor status (estrogen receptor [ER]/progesterone receptor [PgR]) was determined by each study site.

Table 2. Efficacy results

	Dose group		
	40 mg (*n* = 33)	80 mg (*n* = 32)	120 mg (*n* = 32)
Response			
CBR, %	30.3	25.0	25.0
98.3% CI	13.3–52.4	9.5–47.2	9.5–47.2
ORR, %	9.1	9.4	6.3
98.3% CI	1.3–27.9	1.3–28.6	0.4–24.3
CR, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)
PR, *n* (%)	3 (9.1)	3 (9.4)	2 (6.3)
SD ≥ 24 weeks, *n* (%)	7 (21.2)	5 (15.6)	6 (18.8)
SD < 24 weeks, *n* (%)	15 (45.5)	14 (43.8)	13 (40.6)
PD, *n* (%)	8 (24.2)	10 (31.3)	11 (34.4)
Time to progression, months			
Median	4.6	3.7	3.6
95% CI	3.6–5.4	2.1–5.7	1.9–5.6

CBR, clinical benefit rate; CI, confidence interval; CR, complete response; ORR, objective tumor response rate; PD, progressive disease; PR, partial response; SD, stable disease.

Safety. Most patients (72.2%) experienced drug-related AEs (definite, probable, possible) including hot flushes, hyperhidrosis, and nausea as non-hematological toxicities (Table 4). The most common was hot flush, reported by 22.7% of patients. A majority of these AEs observed were mild (grades 1–2) and there was no clear dose dependency regarding severity or frequency of AEs. Discontinuation due to TAS-108-related toxicity was rare (2/97; one patient with grade 3 hypoacusis and one patient with grade 3 dizziness in the 80-mg group). One patient in the 80-mg group had surgery for grade 3 cataracts in both eyes.

Exploratory analysis. Table 5 shows the results of analysis for each value. TAS-108 did not cause significant endometrial thickening (median baseline ET, 3.3 mm; 24 weeks ET, 4.0 mm; $n = 37$). No change was observed in median BMD (−0.30%; $n = 20$), serum I-CTP, and osteocalcin levels. The median triglyceride level decreased significantly from 104.0 to 86.0 mg/dL ($P < 0.0001$); there were no changes in other serum lipids. Increases in PRL, testosterone, and sex hormone-binding globulin levels were observed.

Discussion

This randomized phase II study was designed to evaluate the efficacy and safety of 40, 80, or 120 mg TAS-108 given orally once daily in postmenopausal patients previously treated with one or two regimens of endocrine treatment (with a maximum of one regimen of chemotherapy), with HR-positive MBC, particularly including prior AI- and/or tamoxifen-resistant disease.

As the first step toward the best dose selection, we sought to find the "active" dose level(s) among the three dose groups based on the analysis of the primary end-point. The tolerability at the active dose level(s) was subsequently assessed to achieve a relative balance between efficacy and toxicity of TAS-108. In consequence, it is found that the lower dose of 40 mg showed, numerically, the highest CBR (30.3%) at 24 weeks and met the targeted expectations for clinical activity. This finding suggests that the two higher doses might have been beyond the plateau phase of the dose–response curve and therefore had a potential "reverse dose–response" effect. The safety parameters were similar between the three doses. In addition, secondary efficacy analyses supported the choice because TAS-108 at a dose of 40 mg had similar but slightly higher antitumor activity than the two higher doses. The 40 mg dose of TAS-108 was therefore recommended for further controlled studies against current therapeutic standards. The results observed in this study were largely similar to those reported by Buzdar *et al.*[15]

With the widespread use of AIs in the adjuvant setting, several drugs have recently been reported to be potentially effective in the treatment for breast cancer patients following the failure of AI treatment. Subgroup analysis revealed that there is biological evidence for a CBR of 28.6% (tamoxifen refractory) and 27.1% (AI refractory), and this finding supports the concept that there may be no major cross-resistance between tamoxifen/AI and TAS-108. These encouraging results suggest that this drug can expand the choice of endocrine therapy for MBC patients in that population.

The safety profile of TAS-108 at all dose levels was favorable even when compared with the known safety profile of tamoxifen or other selective estrogen receptor modulators, which was similar to that in a phase I study by Saeki *et al.*[13] The frequent drug-related AEs were hot flush, hyperhidrosis, and nausea, which were of only mild severity (grade 1 or 2), and did not interfere with TAS-108 treatment. The frequency and severity of AEs did not appear to be related to the dose of TAS-108, which has been reported previously in a single-dose study and repeated-dose studies.[10–13] In the present study, grade 3 cataract was reported as a serious AE in one patient aged 63 years. Taking into account the report that tamoxifen can cause visual disorders, the relationship of the cataract to

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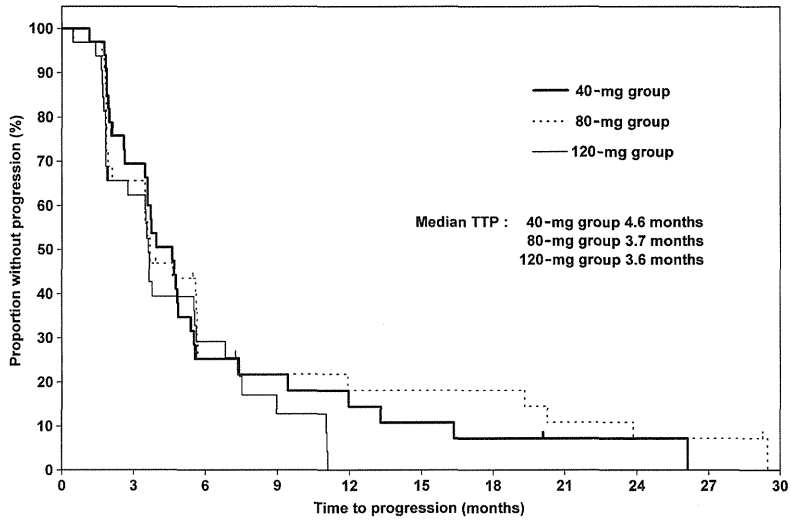

Fig. 1. Kaplan–Meier estimates for time to progression (TTP) in postmenopausal Japanese women with breast cancer treated with three different doses of TAS-108.

Table 3. Subgroup analysis for response to TAS-108 within each dose group (tumor refractory to prior tamoxifen and aromatase inhibitor treatment)

	Tamoxifen refractory (n = 14)	Aromatase inhibitor refractory (n = 70)
Clinical benefit rate (%)	28.6	27.1

TAS-108 was considered "possible". No other clinical observations associated with the significant side-effects of tamoxifen or AIs, such as thromboembolic events or bone fracture, have been reported. Therefore, the low toxicity profile of TAS-108, coupled with the evidence of activity in MBC patients, justifies further clinical testing.

To date, there is no apparent clinical evidence of a stimulating effect of TAS-108 on the endometrium in prior phase I studies involving Japanese and Caucasians patients,[11,13] and in the present exploratory analysis TAS-108 did not cause significant endometrial thickening. In this study, no change was observed in BMD or BMMs, unlike with tamoxifen. This observation suggests that TAS-108 may have few estrogenic effects on bone. Serum triglyceride was significantly decreased with no unfavorable changes in other cardiovascular risk factors tested in this study. TAS-108 had no significant clinical effect on hormones. We acknowledge that because this was not an adjuvant study, there were several limitations to this exploratory analysis, such as a reduction in the number of MBC patients due to withdrawal from this study, and a relatively short length of drug exposure (particularly for analysis of the uterus and bone). Therefore, the effects of TAS-108 on these values seemed tentative and need further investigation. However, the present analysis assessing the clinical potential impact of TAS-108 suggests that this drug may not negatively affect the safety profile of postmenopausal patients.

In conclusion, TAS-108 at the 40 mg dose level showed promising results regarding the primary end-point of this study, and it was well tolerated at all dose levels in postmenopausal Japanese patients who had received one or two previous endocrine therapies. Based on these results, we determined the optimal dose of oral TAS-108 to be 40 mg, once daily, for further clinical studies.

TAS-108, a novel steroidal antiestrogen, may have the potential to develop into a clinically useful second- or third-line endocrine therapy for HR-positive breast cancer refractory to AI and/or tamoxifen.

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Table 4. Drug-related adverse events occurred in >10% of postmenopausal Japanese women with breast cancer treated with TAS-108, in either dose group

Event†	Dose group						Total (n = 97)	
	40 mg (n = 33)		80 mg (n = 32)		120 mg (n = 32)			
	All grades, n (%)	Grades 3–5, n (%)	All grades, n (%)	Grades 3–5, n (%)	All grades, n (%)	Grades 3–5, n (%)	All grades, n (%)	Grades 3–5, n (%)
Hot flush	7 (21.2)	0 (0)	8 (25.0)	0 (0)	7 (21.9)	0 (0)	22 (22.7)	0 (0)
Hyperhidrosis	2 (6.1)	0 (0)	3 (9.4)	0 (0)	6 (18.8)	0 (0)	11 (11.3)	0 (0)
Nausea	1 (3.0)	0 (0)	3 (9.4)	0 (0)	5 (15.6)	0 (0)	9 (9.3)	0 (0)
Uterine leiomyoma	0 (0)	0 (0)	4 (12.5)	0 (0)	1 (3.1)	0 (0)	5 (5.2)	0 (0)
Blood cholesterol increased	4 (12.1)	0 (0)	1 (3.1)	0 (0)	0 (0)	0 (0)	5 (5.2)	0 (0)

†Patients could have had more than one event.

Table 5. Analysis of endometrial thickness (ET), bone mineral density (BMD), bone metabolism markers (BMMs), serum lipids, and endocrine hormones in postmenopausal Japanese women with breast cancer treated with TAS-108

Variable	*n*	Baseline (median)	8† or 24‡ weeks (median)	Change or percentage change§ from baseline to 8† or 24‡ weeks		
				Median	Range	*P*¶
ET, mm	37	3.30	4.00	0.00	−6.00–13.10	0.0850
BMD, g/cm^2	20	0.83	0.84	−0.30	−10.74–8.79	0.5220
BMMs						
Serum Osteocalcin, ng/mL	50	9.50	9.20	−0.62	−38.75–85.42	0.8420
Serum I-CTP, ng/mL	51	3.40	3.40	−3.03	−52.86–105.26	0.4120
Serum lipids						
Total-cho, mg/dL	93	208.00	212.00	0.00	−78.00–94.00	0.4410
HDL-cho, mg/dL	92	62.00	63.90	0.00	−28.00–51.00	0.1960
LDL-cho, mg/dL	91	121.00	127.00	3.00	−57.00–81.00	0.0830
Triglycerides, mg/dL	93	104.00	86.00	−13.00	−217.00–301.00	<0.0001
APO-A1, mg/dL	93	147.00	151.00	4.00	−45.00–59.00	0.1810
APO-B, mg/dL	93	99.00	99.00	1.00	−33.00–42.00	0.6680
Endocrine hormones						
E2, pg/mL	93	10.00	11.00	0.00	−11.00–33.00	0.1310
FSH, mIU/mL	93	44.49	44.80	−2.60	−24.27–26.88	0.3210
Prolactin, ng/mL	93	8.33	8.45	0.42	−41.12–40.79	0.0280
Testosterone, ng/dL	93	0.21	0.22	0.02	−0.23–0.31	0.0190
TSH, μIU/mL	93	2.58	2.51	0.00	−5.90–94.30	0.6350
Cortisol, μg/dL	93	13.60	13.20	0.20	−16.30–22.10	0.7540
SHBG, nmol/L	93	71.40	91.90	14.00	−110.00–133.40	<0.0001

†Bone metabolism markers, serum lipids, and endocrine hormones were assessed at the 8-week point in patients who received TAS-108 for 8 weeks or more. ‡Endometrial thickness and BMD were assessed at the 24-week point in patients who received TAS-108 for 24 weeks or more. §Data are presented as change from baseline, except BMD and BMMs as percentage change from baseline. ¶*P*-values based on the Wilcoxon signed-rank test. APO-A1, apolipoprotein A-I; APO-B, apolipoprotein B; HDL-cho, high-density lipoprotein cholesterol; I-CTP, cross-linked carboxy-terminal telopeptide of type I collagen; LDL-cho, low-density lipoprotein cholesterol; SHBG, sex hormone-binding globulin; Total-cho, total cholesterol; TSH, thyroid-stimulating hormone.

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Disclosure Statement

Hideo Inaji, Takahiro Nakayama, Naohito Yamamoto, Shigehira Saji, Toshiaki Saeki, and Shinzaburo Noguchi have received honoraria from Taiho Pharmaceuticals. Toshiaki Saeki and Shinzaburo Noguchi have received research funding from Taiho Pharmaceuticals. Tadashi Ikeda and Shinzaburo Noguchi have received consulting fees from Taiho Pharmaceuticals. The other authors have declared no conflicts of interest.

Abbreviations

AE	adverse event
AI	aromatase inhibitor
BMD	bone mineral density
BMM	bone metabolism marker
CBR	clinical benefit rate
CEC	Clinical Efficacy Committee
CI	confidence interval
CR	complete response
E2	17β-estradiol
ER	estrogen receptor
ET	endometrial thickness
FSH	follicle-stimulating hormone
HR	hormone receptor
I-CTP	cross-linked carboxy-terminal telopeptide of type I collagen
MBC	metastatic breast cancer
ORR	objective tumor response rate
PR	partial response
PS	performance status
SD	stable disease
TAS-108	(7α)-21-[4-[(diethylamino)methyl]-2-methoxyphenoxy]-7-methyl-19-norpregna-1,3,5(10)-trien-3-ol 2-hydroxy-1,2,3-propanetricarboxylate
TTP	time to progression

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Appendix I

The following institutions participated in this study: Hokkaido Cancer Center (Sapporo), Iwate Medical University (Morioka), Yamagata Prefectural Central Hospital (Yamagata), Tohoku University Hospital (Sendai), KKR Tohoku Kosai Hospital (Sendai), Tochigi Cancer Center (Utsunomiya), Saitama International Medical Center, Saitama Medical University (Hidaka), Saitama Red Cross Hospital (Saitama), Saitama Cancer Center (Ina), National Cancer Center Hospital (Tokyo), St. Luke's International Hospital (Tokyo), Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital (Tokyo), Chiba Cancer Center (Chiba), Tokai University School of Medicine (Isehara), Kanagawa Cancer Center (Yokohama), Yokohama Municipal Citizen's Hospital (Yokohama), Kitasato University School of Medicine (Sagamihara), Niigata Cancer Center Hospital (Niigata), Seirei Hamamatsu General Hospital (Hamamatsu), Aichi Cancer Center (Nagoya), Nagoya Medical Center (Nagoya), Nagoya City University Graduate School of Medical Sciences (Nagoya), Osaka Medical Center for Cancer and Cardiovascular Diseases (Osaka), Graduate School of Medicine, Osaka University (Suita), Osaka National Hospital (Osaka), Osaka Kouseinenkin Hospital (Osaka), Sakai Municipal Hospital (Sakai), Kansai Rosai Hospital (Amagasaki), Hyogo Cancer Center (Akashi), Shikoku Cancer Center (Matsuyama), Hiroshima University Hospital (Hiroshima), Kurashiki Central Hospital (Kurashiki), Kyushu Cancer Center (Fukuoka), and Kumamoto Municipal Hospital (Kumamoto).