Int J Clin Oncol (2006) 11:426–433
DOI 10.1007/s10147-006-0601-6

ORIGINAL ARTICLE

Kazuharu Kai · Reiki Nishimura · Nobuyuki Arima
Haruhiko Miyayama · Hirotaka Iwase

p53 expression status is a significant molecular marker in predicting the time to endocrine therapy failure in recurrent breast cancer: a cohort study

Received: May 13, 2006 / Accepted: June 15, 2006

Abstract
Background. Hormone receptor status has been one of the most important factors in predicting the response to endocrine therapy in breast cancer patients. However, half of those patients with estrogen receptor-positive tumors do not respond to endocrine therapy. There have been no universal factors for predicting resistance to endocrine therapy in this population. Recently, p53 status has been extensively used as a predictive factor for response to systemic therapy, because tumor cells lacking p53 function do not respond to systemic therapy due to a failure in apoptosis. We therefore studied the relationship between the efficacy of endocrine therapy and biological factors, including p53.
Methods. The expression of p53, Ki67, and human epidermal growth factor receptor (HER)2 was examined by immunostaining in the primary tumors of 53 patients who received endocrine therapy for recurrent or advanced breast cancer. The following clinical factors were also analyzed: site treated, disease-free interval, and response to first-line endocrine therapy. To evaluate the significance of these factors, time to endocrine therapy failure (TTEF), or the total duration of sequential endocrine therapies was adopted as representing the clinical outcome.
Results. The median TTEF was 16.1 months (range, 2.5–89.9 months). Multivariate analysis showed significantly reduced TTEF associated with no response to first-line endocrine therapy ($P = 0.006$ and $P = 0.002$ in all patients and in recurrent patients, respectively) and associated with positive p53 expression ($P = 0.066$ and $P = 0.004$, respectively).
Conclusion. p53 expression status was a significant molecular marker as well as the response to first-line endocrine therapy for predicting TTEF in recurrent breast cancer with hormone-sensitive disease.

Key words Time to endocrine therapy failure · Response to first-line endocrine therapy · p53 expression status

Introduction

Hormone receptor status has been one of the most important factors in predicting the response to endocrine therapy for breast cancer patients.[1] However, half of these patients with estrogen receptor (ER)-positive tumors do not respond to endocrine therapy.[2,3] Previous investigators have researched mechanisms of resistance to endocrine therapy and have attempted to identify reliable factors for predicting such resistance in patients with ER-positive tumors.[3,4] Recently, human epidermal growth factor receptor (HER)2 has been regarded as one of the most reliable predictors in that population; however, its significance varies according to the therapeutic endocrine compounds used. For example, HER2 overexpression is relevant to resistance to tamoxifen[3,5] but is not relevant for resistance to the third-generation aromatase inhibitors (AIs).[6,7]

Recently, p53 expression status has been extensively used as a predictive factor for response to systemic therapy,[8,9] because tumor cells with nonfunctional p53 do not respond to systemic therapy due to a failure in apoptosis.

We therefore studied the relationship between the efficacy of endocrine therapy and biological factors, including p53, as well as Ki67 and HER2. In addition to these biological factors, we also examined the significance of clinical factors that have already been accepted in practice, including the disease-free interval (DFI), response to first-line endocrine therapy (RFET), and the site treated.

K. Kai (✉) · H. Iwase
Breast and Endocrine Surgery, Faculty of Medical and Pharmaceutical Science, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan
Tel. +81-96-373-5521; Fax +81-75-761-3441
e-mail: 20105995@fc.kuh.kumamoto-u.ac.jp

K. Kai · R. Nishimura
Breast and Endocrine Surgery, Kumamoto City Hospital, Kumamoto, Japan

N. Arima · H. Miyayama
Clinical Pathology, Kumamoto City Hospital, Kumamoto, Japan

To evaluate the significance of these factors, we adopted time to endocrine therapy failure (TTEF), or the total duration of sequential endocrine therapies without interruption by chemotherapy, radiotherapy, or surgery, as representing the clinical outcome. The TTEF is as important as clinical response to endocrine therapy, because it directly affects the quality and duration of life. Therefore, we introduced it as an index of efficacy of endocrine therapy and studied factors that were predictive for it.

Patients and methods

Study cohort and characteristics

The study cohort consisted of 53 patients with advanced or recurrent breast cancer. These patients were treated with endocrine compounds at Kumamoto City Hospital, Kumamoto, Japan, between October 1984 and December 2004. The research protocol for this study was approved by the Ethics Committee of Kumamoto City Hospital. Informed consent was obtained from all patients before entering this study. The patients' characteristics are shown in Table 1. The mean age was 55.5 years (range, 38–75 years). Of all 53 patients, 23% were premenopausal and 77% were postmenopausal; 91% (48/53) of the patients had ER- and/or progesterone receptor (PgR)-positive tumors, whereas there were no patients with ER- and PgR-negative tumors. In the remaining 5 patients (9%), the ER and PgR status of the tumors was unknown; 4 of these 5 patients had recurrent disease, and 1 had advanced disease. The 4 patients with recurrent disease had long DFIs (median, 88.0 months; range, 59–132 months).

The cancer was recurrent in 47 patients, and advanced in 6 patients. All of the recurrent patients had undergone surgical treatment for primary breast cancer (either mastectomy or lumpectomy). After surgery, 2% of the 47 recurrent patients received no additional therapy. and, in 6% of these 47 patients, details of additional therapy were not known. Of the remaining recurrent patients, 21% received systemic adjuvant therapy consisting of endocrine therapy alone, 9% received chemotherapy alone, and 62% received combined endocrine therapy and chemotherapy. Patients who had axillary lymph node involvement had received chemotherapy with either 5-fluorouracil derivatives for 2 years or a combination of cyclophosphamide, methotrexate, and fluorouracil (CMF) if treated before April 1999, and if treated after May 1999, they received epirubicin and cyclophosphamide (EC). However, 1 patient with marked lymph node involvement received paclitaxel following EC as adjuvant therapy. All 6 patients with advanced disease had distant metastasis at the start of the endocrine therapy.

Before the first-line endocrine therapy, 36% (19/53) of the patients with recurrent or advanced disease had received chemotherapies. In these 19 patients, the profiles of prior chemotherapy were as follows: anthracycline-containing chemotherapy, 6; taxane-containing chemotherapy, 5; sequential anthracycline and taxanes, 4; and chemotherapy based on oral fluorouracil, 4.

Treatment and follow up

All patients were treated with endocrine compounds sequentially according to an algorithm described previously.[10] After entering this study, all of the participants took an antiestrogen (tamoxifen or toremifen), a luteinizing hormone-releasing hormone analogue, a third-generation AI (anastrozole, exemestane, or letrozole), a second-generation AI (fadrozole), or medroxyprogesterone acetate. At the start of the endocrine therapy, 23% (12/53) of the patients were premenopausal. However, all of the premenopausal patients experienced menopause during the endocrine therapy. Therefore, all of the 53 patients received a third-generation AI at any level of treatment (i.e., first-line, second-line, etc; Table 2).

In addition to endocrine intervention, bisphosphonates were administered to patients with bone metastases when the disease progressed or when the patients complained of bone pain. The sequences of and changes in endocrine compounds were decided according to the physicians' discretion on the basis of menopausal status. The details of the clinical courses and clinicopathological factors in the patients were recorded in a prospective database after the initial administration of the first-line endocrine compound. Patients were observed for disease progression and treatment failure at least once every 4 weeks after the start of the endocrine therapy. We recorded the time from the start of the endocrine therapy to the interruption of sequential endocrine therapies or follow-up discontinuance, and calculated the TTEF by the Kaplan-Meier method. Information on the patients before the first-line endocrine therapy was investigated from medical charts. The median follow-up duration was 24.8 months (range, 3.2–117.1 months).

Evaluation of response to endocrine therapy

Measurable disease was defined as the presence of bidimensionally or unidimensionally measurable lesions as determined by physical examination, ultrasound, or radiographic scan. Osteolytic bone lesions were considered measurable. Single metastatic lesions smaller than 0.5 cm, malignant pleural effusions or ascites, positive bone scan, and purely osteoblastic or intratrabecular bone lesions were not classified as measurable disease. Lesions not classified as measurable constituted nonmeasurable but assessable disease.

The objective response to endocrine therapy was assessed clinically according to criteria of the International Union Against Cancer.[11] Objective responses were classified as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) for both measurable and nonmeasurable disease. A best response of SD was only assigned when responses of SD or better were observed for at least 24 weeks. Responders were those patients with a best objective response of CR or PR. Patients

Table 1. Clinical and biological characteristics of patients with recurrent or advanced breast cancer

Characteristics	Number of patients (%)
Total number of patients	53 (100)
Age at the start of endocrine therapy (years; mean, 55.5)	
≤50	16 (30)
>50	37 (70)
Range	38 to 75
Menopause at the start of endocrine therapy	
Premenopausal	12 (23)
Postmenopausal	41 (77)
Disease setting	
Recurrent	47 (89)
Advanced	6 (11)
Hormone receptor	
ER and/or PgR-positive	48 (91)
ER and PgR-negative	0 (0)
ER and PgR unknown	5 (9)
Ki67	
Negative	17 (33)
Positive	35 (67)
p53	
Negative	37 (73)
Positive	14 (27)
HER2	
Negative	40 (78)
Positive	11 (22)
Site treated	
Soft tissue	15 (28)
Bone	16 (30)
Viscera	22 (42)
Disease-free interval (months; mean, 61.5)[a]	
≤24	12 (26)
>24	35 (74)
Adjuvant therapy[a]	
Endocrine therapy	10 (21)
Chemotherapy	4 (9)
Combined	29 (62)
Not done	1 (2)
Unknown	3 (6)
Prior chemotherapy for recurrent or advanced diseases	
Done	19 (36)
Not-done	31 (58)
Unknown	3 (6)

Ki67 data were available for 52 samples, and p53 and HER2 data were available for 51 samples
Dichotomized values were used
ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2
[a] Only in recurrent patients

Table 2. Therapeutic course and efficacy of endocrine therapy in patients with recurrent or advanced breast cancer

	Number of patients (%)					
	Level of treatment					
	First-line	Second-line	Third-line	Fourth-line	Fifth-line	Total
No of patients at each level	53 (100)	38 (100)	23 (100)	7 (100)	3 (100)	
Treatment with third-generation AI	31 (58)	22 (58)	10 (43)	3 (43)	2 (67)	53 (100)
Response to endocrine therapy[a]	19 (36)	5 (13)	1 (4)	1 (14)	0 (0)	
Achievement of clinical benefit[b]	34 (64)	13 (34)	3 (13)	2 (29)	1 (33)	

[a] Sum of confirmed complete responses (CRs) and partial responses (PRs)
[b] Sum of confirmed CRs, PRs and confirmed stable diseases (SDs), SDs lasting for 24 weeks, AI, aromatase inhibitor

with clinical benefit were defined as those responding (CR + PR) plus those with SD for at least 24 weeks. The objective response was judged independently by two physicians. If they judged differently, a third opinion was sought for a final judgment.

Immunohistochemical analysis

Operative or biopsy specimens were fixed in buffered formalin and embedded in paraffin wax. One 4-μm section from each submitted paraffin block was first stained with hematoxylin and eosin in order to verify that an adequate number of invasive ductal carcinoma cells were present and that the quality of fixation was sufficient for immunohistochemical analysis. Serial sections (4-μm) were prepared from selected blocks and float-mounted on adhesive-coated glass slides for estrogen receptor (ER) α, progesterone receptor (PgR), Ki67, p53, and HER2 staining. Primary antibodies included mouse monoclonal antihuman ER a antibody (DAKO Glostrup, Denmark) at 1:75 dilution for ER a, mouse monoclonal antihuman progesterone receptor (PgR) antibody (DAKO) at 1:700 dilution for PgR, monoclonal mouse antihuman Ki67 antibody (DAKO) at 1:50 dilution for Ki67, monoclonal mouse antihuman p53 protein antibody (Japan Tanner, Osaka, Japan) at 1:50 dilution for p53, and humanized antihuman HER2/neu oncoprotein antibody (DAKO; HercepTest) for HER2. The DAKO EnVision system (DAKO EnVision labeled polymer, peroxidase) was used as the detection system for ER α, PgR, p53, and Ki67.

Immunohistochemical scoring

Immunostained slides were scored after the entire slide had been evaluated by light microscopy. The expressions of ER and PgR were scored according to the proportion of positive-stained cells. Any brown nuclear staining in invasive breast epithelium was counted as positive staining. Tumors in which the proportion of positive-stained cells was 10% or greater were considered to be positive for ER and PgR expression. HER2 immunostaining was evaluated with the Hercep Test (DAKO). To determine the score for HER2 expression, the membrane staining pattern was estimated and given a score of 0 to 3+.

Tumors with scores of 2 or greater were considered to be positive for HER2 overexpression. The expression status of p53 and Ki67 was assessed according to the estimated proportion of nuclear staining in tumor cells that were positively stained. Scoring criteria for p53 were as follows: (score, proportion of nuclear staining; none, 0; <1/20, 1; 1/20–1/2, 2; and >1/2, 3). Scoring criteria for Ki67 were as follows (score, proportion of nuclear staining: none, 0; <1/5, 1; 1/5–1/2, 2; and >1/2, 3). Tumors with a score of 3 for p53 were considered to be positive for p53 expression, and tumors with a score of 2 or 3 for Ki67 were considered to be positive for Ki67 expression. In this study, Ki67 data were available for 52 samples, and p53 and HER2 data were available for 51 samples.

Statistical methods

Estimation of TTEF was performed by the Kaplan-Meier method, and differences between TTEF curves were assessed with the log rank test. Cox's proportional hazards model was used for univariate and multivariate analyses of factors predictive for TTEF. A two-sided P value of <0.05 was considered statistically significant.

Results

Treatment compounds and efficacy of endocrine therapy

With regard to treatment compounds used, 58% (31/53), 58% (22/38), and 43% (10/23) of the patients were treated with third-generation AIs at first, second, and third-line endocrine therapy, respectively (Table 2). The response rates for endocrine therapies were 36%, 13%, and 4%, at first, second, and third-line endocrine therapy, respectively (Table 2). The median TTEF was 16.1 months (range, 2.5–89.9 months). Of all the 53 patients, 18 were still responding to endocrine therapy at the end of this study. In the remaining 35 patients, ultimate endocrine therapy failure was observed during the follow-up period. Among these 35 patients, 17 had PD in measurable visceral lesions; 7 were receiving bisphosphonate or irradiation for symptomatic bone lesions, or had PD in bone lesions; 4 patients had PD at local sites; 6 patients had tumor marker elevation without apparent PD; and 1 patient had a new brain metastasis. No patients refused endocrine therapy owing to compound-related toxicities or other factors during the follow-up period.

Relationship between time to endocrine therapy failure (TTEF) and clinicopathological factors

Positivity for all the molecular markers tested (Ki67, p53, HER2) was associated with significantly reduced TTEF. P values for Ki67, p53, and HER2 were 0.047, 0.005, and 0.034, respectively (Fig. 1). In regard to clinical factors, no response to first-line endocrine therapy failure (RFET) was associated with significantly reduced TTEF (P = 0.011). On the other hand, there was no relationship between sites treated or DFI and TTEF (P = 0.326 and 0.190, respectively).

Predictive analysis for time to endocrine therapy failure (TTEF)

On univariate analysis (Table 3), Ki67 status (P = 0.047), p53 status (P = 0.005), and HER2 status (P = 0.034), as well as RFET (P = 0.011) were strongly able to predict TTEF. On multivariate analysis, the site treated was excluded for the prediction of TTEF, because there was thoroughly no relationship between site treated and TTEF on the univariate analysis. Multivariate analysis in all patients

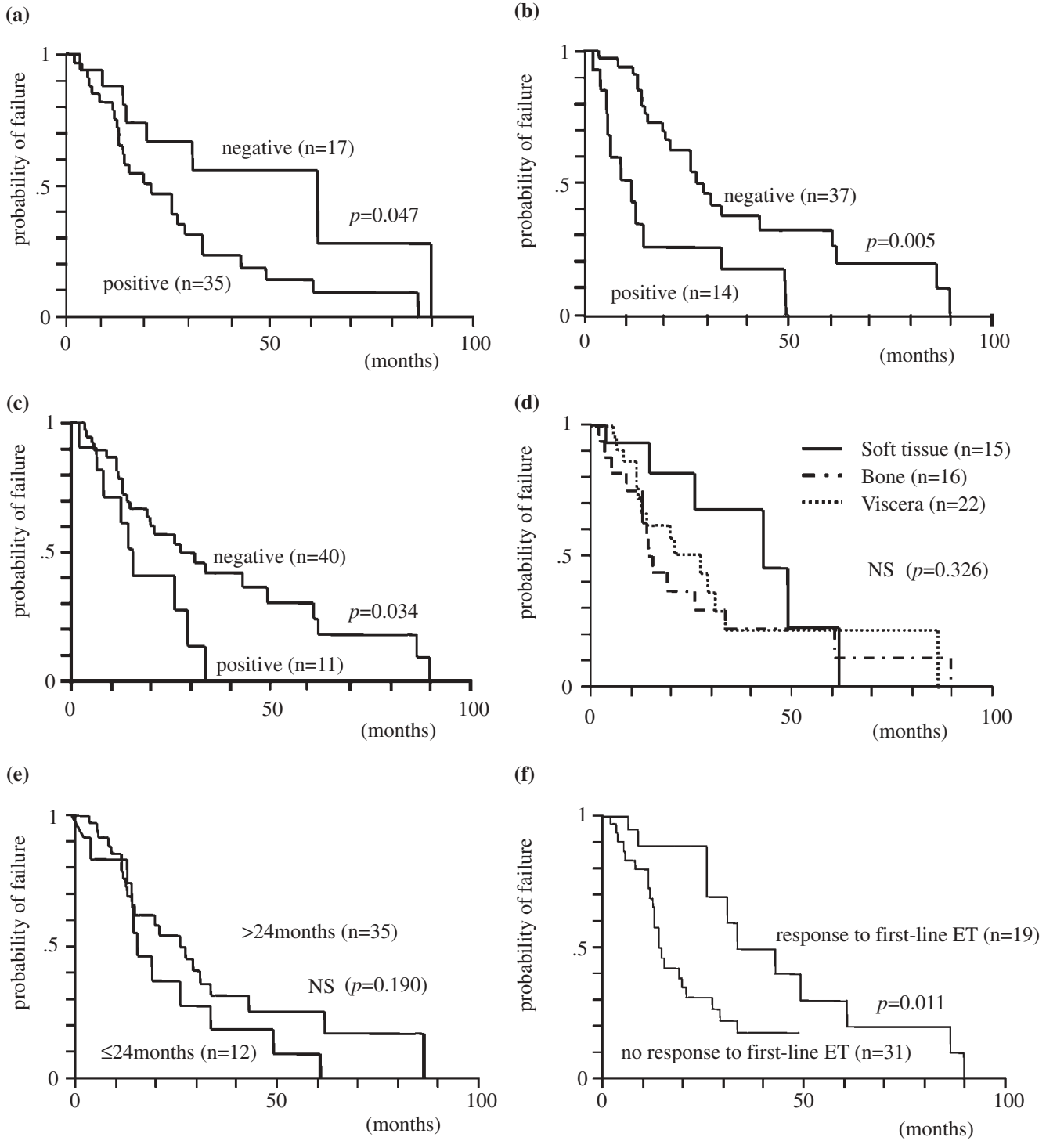

Fig. 1a-f. Relationship between time to endocrine therapy failure (TTEF) and clinicopathological factors in 53 patients with recurrent or advanced breast cancer with hormone-responsive disease. TTEF was significantly longer in patients whose tumors were negative for **a** Ki67, **b** p53, or **c** human epidermal growth factor receptor 2 (HER2) than in patients whose tumors were positive for Ki67 ($P = 0.047$), p53 ($P = 0.005$), or HER2 ($P = 0.034$), respectively. In regard to clinical factors, there were no relationships between **d** sites treated or **e** disease-free interval (DFI) and TTEF ($P = 0.326$ or 0.190, respectively). On the other hand, **f** TTEF was significantly longer in patients who responded to first-line endocrine therapy (*ET*) than in patients who did not respond to first-line endocrine therapy ($P = 0.011$). *NS*, not significant

showed reduced TTEF associated with no RFET (P = 0.006) and with positive p53 status, although this association did not quite reach significance (P = 0.066). On multivariate analysis in recurrent patients only, positive p53 status (P = 0.004) and no RFET (P = 0.002) were significantly predictive for reduced TTEF. There was no significant relationship between TTEF and Ki67 or HER2 either in all patients or in the recurrent patients, and there was no significant

relationship between TTEF and DFI in the recurrent patients.

Discussion

We investigated the expression of p53, Ki67, and HER2 in primary breast tumor specimens as molecular markers, and DFI and RFET as clinical factors in predicting TTEF, in 53 patients with recurrent or advanced breast cancer who received endocrine therapy without concurrent therapies. Also, we introduced TTEF as an index of efficacy for endocrine therapy. Our results indicate that p53 expression status and response to first-line endocrine therapy are strongly predictive of TTEF in recurrent or advanced breast cancer.

Time to treatment failure (TTF) is as important as clinical response in evaluating the efficacy of endocrine therapy. TTF generally reflects the quality and duration of life in patients with advanced or recurrent breast cancer.[12–14] We therefore introduced TTEF as an indicator for the outcome of endocrine therapy, which might better reflect the quality and duration of life than other outcomes, such as overall survival. It can be considered as reasonable to turn our attention from time to death to time to endocrine therapy failure in evaluating the outcome of endocrine therapy.

In the present study, the cohort were expected to respond to endocrine therapy because most of the patients were judged positive for hormonal receptor or had a long DFI (Table 1). However, in general, some patients, even those with ER-positive tumors, do not respond to endocrine therapy.[2] There have been no ideal predictors for the efficacy of endocrine therapy in this population. From this point of view, the essence of the present study was to identify predictors of resistance to endocrine therapy among those who were considered to be responsive to it. We found that the p53 expression status and RFET were significant predictors of TTEF in such a cohort. Kurebayashi et al.[15] indicated that the RFET was the best predictive factor for response to second-line endocrine therapy in patients with hormonal receptor-positive tumors or long DFI. We also showed its significance in predicting TTEF. Undoubtedly, the response to first-line endocrine therapy can be a useful and appropriate factor for predicting the efficacy of endocrine therapy, especially in practical medicine. However, we cannot predict TTEF at the start of first-line endocrine therapy. Therefore, p53 status can be an important factor in predicting TTEF at the start of first-line endocrine therapy.

Table 3. Univariate Cox regression model analysis of time to endocrine therapy failure in patients with recurrent or advanced breast cancer

Parameter	Time to endocrine therapy failure	
	OR (95% CI)	*P* value
Ki67		
Negative	1	0.047
Positive	2.3 (1.0–5.4)	
P53		
Negative	1	0.005
Positive	2.9 (1.4–6.2)	
HER2		
Negative	1	0.034
Positive	2.4 (1.1–5.4)	
Site treated		
Soft tissue	1	0.326
Bone	2.0 (0.8–5.4)	
Viscera	1.7 (0.6–4.3)	
Disease-free interval (months)		
>24	1	0.190
≤24	1.6 (0.8–3.4)	
Response to first-line ET		
Response	1	0.011
No response	3.1 (1.3–7.4)	

CI, confidence interval; OR, odds ratio; ET, endocrine therapy

Table 4. Multivariate Cox regression model analysis of time to endocrine therapy failure in patients with recurrent or advanced breast cancer

Parameter	TTEF (all patients)		TTEF (recurrent patients)	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Ki67				
Negative	1	0.16	1	0.218
Positive	2.1 (0.7–6.1)		2.0 (0.7–6.1)	
p53				
Negative	1	0.066	1	0.004
Positive	2.2 (0.9–5.0)		4.6 (1.6–13.4)	
HER2				
Negative	1	0.175	1	0.726
Positive	1.8 (0.8–4.5)		1.2 (0.5–3.0)	
Disease-free interval (months)				
>24			1	0.806
≤24			1.1 (0.4–3.2)	
RFET				
Response	1	0.006	1	0.002
No response	3.6 (1.4–9.0)		7.5 (2.2–25.8)	

TTEF, time to endocrine therapy failure; CI, confidence interval; OR, odds ratio; RFET, response to first-line endocrine therapy failure

Previous reports have presented varying views about the correlation between response to endocrine therapy and p53 status. Some investigators reported that there was no correlation between them,[16,17] while others reported the significance of p53 status in predicting response to endocrine therapy.[8,18–22] These varying opinions may be caused by the following factors: first, differences between studies in the characteristics of patients and population sizes; second, differences in methods used to evaluate p53 status (immunostaining, cDNA sequencing, and so on); and third, differences in the thresholds used for evaluating p53 expression status. With regard to methods of evaluating p53 status, the reliability of p53 status, measured by immunostaining, in predicting response to endocrine therapy is controversial because of the discordant results reported, as described above. However, in all studies using cDNA sequencing to detect mutations in the *p53* gene, *p53* mutations were significantly predictive of resistance to endocrine therapy.[8,18] In addition, Berns et al.[8] showed that 20% of tumors with a mutant *p53* gene did not have p53 protein accumulation in nuclei of tumor cells. Conversely, there is a possibility of p53 protein accumulation in nuclei even if there is no mutation in the *p53* gene. On the basis of these reports, we may speculate that the results based on cDNA sequencing are more reliable than those based on immunostaining.

In the present study, p53 status was assumed to be positive when nuclear staining was seen in more than 50% of tumor cells. This threshold is higher than that reported by other researchers. Therefore, the high expression of p53 in our study may be considered to reflect mutations of the *p53* gene more precisely. Consequently, there may be fewer false-positive cases in our data than in previous reports. Actually, there was no relationship between p53 status and TTEF, using the threshold between score 1 and score 2 for p53 status (data not shown). In a previous study, Elledge. et al.[17] set up a threshold of 10% for the proportion of positive cells by immunostaining, but could not show a correlation between p53 protein accumulation in nuclei and resistance to tamoxifen. These findings indicate that setting up a threshold for p53 status in immunostaining can be central in deciding the significance of p53 status. In the present study, the rate of p53 positivity was 27% (14/51). As we could predict early endocrine therapy failure in about a quarter of our patients, we could regard our threshold of p53 status as reasonable.

At first-line endocrine therapy, 31 patients were treated with third-generation AIs. Among these 31 patients, those with p53-positive tumors had a significantly shorter TTF than those without p53-positive tumors, although the significance was proven only by univariate analysis (data not shown). This finding indicates that p53 expression status may be a universal factor for predicting the efficacy of endocrine therapy, irrespective of the therapeutic endocrine compound used, in contrast to HER2 expression status.[6,7]

In conclusion, the present study indicated that p53 expression status and RFET were significant predictive factors for TTEF in hormone-sensitive recurrent or advanced breast cancer. In particular, p53 expression status could be an important molecular marker for predicting TTEF at the start of first-line endocrine therapy.

Acknowledgments We thank Hiroko Yamashita, Zhenhuan Zhang, and Yutaka Yamamoto for helping to draft the manuscript.

References

1. Ciocca DR, Elledge R (2000) Molecular markers for predicting response to tamoxifen in breast cancer patients. Endocrine 13:1–10
2. Kardinal CG (2002) Hormonal and endocrine therapy of breast cancer. In: Donegan WL, Spratt JS (eds) Cancer of the breast, fifth edn. Elsevier Science, St. Louis, pp 693–737
3. Ring AE, Ellis PA (2002) Predictors of response to systemic therapy in breast cancer. Forum Genova 12:19–32
4. Osborne CK, Shou J, Massarweh S, et al. (2005) Crosstalk between estrogen receptor and growth factor receptor pathways as a cause for endocrine therapy resistance in breast cancer. Clin Cancer Res 11:865–870
5. Arpino G, Green SJ, Allred DC, et al. (2004) HER2 amplification, HER-1 expression, and tamoxifen response in estrogen receptor-positive metastatic breast cancer: a Southwest Oncology Group study. Clin Cancer Res 10:5670–5676
6. Ellis MJ, Coop A, Singh B, et al. (2001) Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. J Clin Oncol 19:3808–3816
7. Dixon JM, Jackson J, Hills M, et al. (2004) Anastrozole demonstrates clinical and biological effectiveness in oestrogen receptor-positive breast cancers, irrespective of the erbB2 status. Eur J Cancer 40:2742–2747
8. Berns EM, Foekens JA, Vossen R, et al. (2000) Complete sequencing of TP53 predicts poor response to systemic therapy of advanced breast cancer. Cancer Res 60:2155–2162
9. Fitzgibbons PL, Page DL, Weaver D, et al. (2000) Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 124:966–978
10. Hortobagyi GN (1998) Treatment of breast cancer. N Engl J Med 339:974–984
11. Hayward JL, Carbone PP, Heuson JC, et al. (1977) Assessment of response to therapy in advanced breast cancer: a project of the Programme on Clinical Oncology of the International Union Against Cancer, Geneva, Switzerland. Cancer 39:1289–1294
12. de Haes H, Olschewski M, Kaufmann M, et al. (2003) Quality of life in goserelin-treated versus cyclophosphamide + methotrexate + fluorouracil-treated premenopausal and perimenopausal patients with node-positive, early breast cancer: the Zoladex Early Breast Cancer Research Association Trialists Group. J Clin Oncol 21:4510–4516
13. Piccart M, Parker LM, Pritchard KI (2003) Oestrogen receptor downregulation: an opportunity for extending the window of endocrine therapy in advanced breast cancer. Ann Oncol 14:1017–1025
14. Shimozuma K, Sonoo H, Ichihara K (1995) Analysis of the factors influencing the quality of life of patients with advanced or recurrent breast cancer. Surg Today 25:874–882
15. Kurebayashi J, Sonoo H, Inaji H, et al. (2000) Endocrine therapies for patients with recurrent breast cancer: predictive factors for responses to first- and second-line endocrine therapies. Oncology 59:31–37
16. Archer SG, Eliopoulos A, Spandidos D, et al. (1995) Expression of ras parn21, p53 and c-erbB-2 in advanced breast cancer and response to first line hormonal therapy. Br J Cancer 72:1259–1266
17. Elledge RM, Green S, Howes L, et al. (1997) bcl-2, p53, and response to tamoxifen in estrogen receptor-positive metastatic breast cancer: a Southwest Oncology Group study. J Clin Oncol 15:1916–1922

18. Bergh J, Norberg T, Sjogren S, et al. (1995) Complete sequencing of the p53 gene provides prognostic information in breast cancer patients, particularly in relation to adjuvant systemic therapy and radiotherapy. Nat Med 1:1029–1034
19. Silvestrini R, Benini E, Veneroni S, et al. (1996) p53 and bcl-2 expression correlates with clinical outcome in a series of node-positive breast cancer patients. J Clin Oncol 14:1604–1610
20. Berns EM, Klijn JG, Look MP, et al. (2003) Combined vascular endothelial growth factor and TP53 status predicts poor response to tamoxifen therapy in estrogen receptor-positive advanced breast cancer. Clin Cancer Res 9:1253–1258
21. Berns EM, Klijn JG, van Putten WL, et al. (1998) p53 protein accumulation predicts poor response to tamoxifen therapy of patients with recurrent breast cancer. J Clin Oncol 16:121–127
22. Horne GM, Anderson JJ, Tiniakos DG, et al. (1996) p53 protein as a prognostic indicator in breast carcinoma: a comparison of four antibodies for immunohistochemistry. Br J Cancer 73:29–35