〈Regular Article〉

Predictors for the outcomes of patients with estrogen receptor-positive, HER2-negative advanced breast cancer treated using palbociclib plus endocrine therapy

Yutaka YAMAMOTO [1)], Ryohei OGATA [1)], Naoki KANOMATA [2)], Emi KISHINO [1)], Takuya MORIYA [3)], Junichi KUREBAYASHI [1)]

1) Department of Breast and Thyroid Surgery, Kawasaki Medical School,
2) Department of Pathology, St. Luke's International Hospital,
3) Department of Pathology, Kawasaki Medical School

ABSTRACT A cyclin-dependent kinase (CDK) 4/6 inhibitor, palbociclib (PAL), combined with endocrine therapy is frequently used for the treatment of patients with estrogen receptor (ER)-positive, human epidermal growth factor receptor (HER) 2-negative advanced breast cancer. However, as predictors for the outcomes remain unclear, we retrospectively investigated them.

A total of 36 patients with ER-positive, HER2-negative advanced breast cancer were treated using PAL plus endocrine therapy at our hospital. Treatment outcomes, objective response rates (ORR), progression-free survival (PFS) and post-treatment overall survival (OS) were analyzed. As possible predictive biomarkers, retinoblastoma protein (Rb), phosphorylated Rb (pRb) and different CDKs were immunohistochemically investigated using primary tumor tissues. Non-visceral metastasis, use of fulvestrant (FUL) and the 1st- or 2nd-line treatment were significant predictors for a better ORR (P = 0.0080, P = 0.0080 and P = 0.0080, respectively). No objective response (OR) was observed in patients with progesterone receptor (PR)-negative, CDK6-positive or cytosolic cyclin E1-positive tumors. Non-visceral metastasis and use of FUL were significant predictors for a better PFS (P = 0.0030 and P = 0.0443, respectively). The Cox proportional hazards model revealed that visceral metastasis (hazard ratio [HR], 4.9; P = 0.0019) and PR-negativity (HR, 3.2; P = 0.0411) were independent predictors for a poorer PFS. Non-visceral metastasis, pRb-negativity and CDK6-negativity were significant predictors for a better OS (P = 0.0281, P = 0.0014 and P = 0.0396, respectively). The Cox proportional hazards model revealed that visceral metastasis (HR, 7.2; P = 0.0131) and pRb-positivity (HR, 18.5; P = 0.0060) were independent predictors for a poorer OS.

In conclusion, PR-negativity and pRb-positivity in primary tumors may be independent

Corresponding author
Junichi Kurebayashi
Department of Breast and Thyroid Surgery, Kawasaki Medical School, 577 Matsushima, Kurashiki, 701-0192, Japan
Phone : 81 86 462 1111
Fax : 81 86 462 1199
E-mail: kure@med.kawasaki-m.ac.jp

predictors for PFS and OS, respectively. CDK6-positivity and cytosolic cyclin E1-positivity may be predictors for a poorer OS and ORR, respectively. Further investigation is needed to confirm these factors.

doi：10.11482/KMJ-E202046135 *(Accepted on August 28, 2020)*

Key words：Palbociclib, Predictors, Response, Progression-free survival, Overall survival

INTRODUCTION

Cyclin-dependent kinase (CDK) 4/6 inhibitors, such as palbociclib (PAL), combined with endocrine therapy are markedly beneficial for patients with hormone receptor (HR)-positive, human epidermal growth factor receptor (HER) 2-negative advanced breast cancer. Recent clinical trials revealed that the addition of these CDK 4/6 inhibitors to endocrine therapy significantly improved progression-free survival (PFS) and overall survival (OS) compared with endocrine therapy alone. These studies prompted physicians to commonly use the combination of a CDK 4/6 inhibitor and endocrine therapy for the treatment of patients with HR-positive HER2-negative advanced breast cancer. However, *de novo* and acquired resistance to this combined therapy is frequently observed and the molecular mechanisms of action responsible for such resistance remain to be fully elucidated[1)].

A large number of preclinical and clinical studies have explored key molecules responsible for the responsiveness and resistance to CDK 4/6 inhibitors. Alteration of cell cycle machinery, such as dysfunction or loss of retinoblastoma protein (Rb), amplification of the CDK6 gene and up-regulation of the cyclin E1/CDK2 pathway, was suggested to induce resistance. Hyper-activation of growth factor receptor pathways, such as fibroblast growth factor receptor (FGFR) and HER 3 pathways, were suggested to induce resistance. A down-stream signaling pathway of the growth factor receptor pathways, such as the PI3K/AKT/mTOR pathway, was also suggested to induce resistance. These possible biomarkers were mainly found in preclinical studies[2)]. Several translational studies using clinical samples demonstrated that alteration of the cell cycle machinery, such as mutations of the Rb gene, CDK6 amplification and up-regulation of growth factor receptor pathways, was uncommon in primary breast tumors and metastatic tumor cells in the blood and recurrent sites[3-7)]. Thus, there are no clinically applicable predictors for responsiveness and resistance to the combined therapy.

To explore the clinical and biological factors influencing the outcomes of patients with advanced breast cancer treated by combined therapy, we conducted this retrospective study. Expression levels of possible biological factors, Rb, phosphorylated Rb and different CDKs, were immunohistochemically evaluated in primary breast tumors. The relationships among clinical factors, biological factors and clinical outcomes (objective response rate [ORR], PRS and OS) were analyzed.

SUBJECTS AND METHODS

Patients and treatments

A total of 42 patients with advanced breast cancer were treated using PAL plus endocrine therapy between February 2018 and December 2019 at the Department of Breast and Thyroid Surgery, Kawasaki Medical School Hospital. Two patients with synchronous bilateral breast cancer and one patient with metachronous bilateral breast cancer were excluded. Four patients were diagnosed with HR-positive, HER2-negative tumors in metastatic lesions and excluded from this study. One patient who had a synchronous bilateral breast cancer was diagnosed with an HR-positive, HER2-negative tumor in a metastatic lesion. Tumor tissue samples obtained from core needle

biopsy (CNB) or surgically resected tumors were available for 36 patients. The eligibility criteria of this study were advanced breast cancer patients whose primary tumor samples were available for immunohistochemical (IHC) analysis.

PAL (125 mg/body) was administered orally on days 1 to 21 of a 28-day cycle together with aromatase inhibitors every day or FUL (500 mg/month with a loading dose). Due to adverse events or patient intolerance, doses of PAL were reduced during or from the beginning of the treatment in 18 patients. Luteinizing hormone-releasing hormone was also administered to six premenopausal patients.

The median age of the patients was 61 years (range: 42-90). All patients were female. The Eastern Cooperative Oncology Group performance status was 0 in 16 patients, 1 in 19 and 2 in 1. Fifteen had recurrent diseases and 11 had stage IV diseases. Target lesions were soft tissues in 21 patients, bones in 23, the lungs in 17 and the liver in 14. The median number of previous endocrine therapies was 3 (range: 1-10). Long-stable disease (L-SD, SD for over 6 months) was achieved by the previous endocrine therapy in 18. The line of treatment for PAL therapy was the 1st or 2nd in 15 patients and the 3rd or later in 21. Chemotherapeutic agents were administered as adjuvant or recurrence therapy before PAL therapy in 18. Due to serious adverse effects, such as neutropenia and liver dysfunction, PAL therapy was discontinued in three patients. The median follow-up time was 16.5 months (range: 2-26). The background characteristics of the patients are summarized in Table 1.

IHC analysis

As CNB samples were fixed more appropriately for immunostaining, IHC results using the CNB samples were adopted for analysis in this study.

Expression levels of the estrogen receptor (ER) and progesterone receptor (PR) were evaluated using the anti-ER monoclonal antibody 1D5 (1:50 dilution; Dako, Glostrup, Denmark) and anti-PR monoclonal antibody PgR636 (1:800 dilution; Dako), respectively. The cut-off value for both ER and PR positivity was 1%. HER2 expression was assessed by IHC using HercepTest (Dako). The results were evaluated according to the criteria of

Table 1. Background characteristics of the study subjects

Age (years)	42 - 90 (median, 61)	
PS	0	16
	1	19
	2	1
Diseases	Stage IV	11
	Recurrence	15
Target sites	Soft tissues	21
	Bones	23
	Lungs	17
	Liver	14
Number of previous endocrine therapies	1 - 10 (median, 3)	
Effects of previous endocrine therapy	L-SD	18
	SD or PD	18
First- or 2nd-line use	Yes	15
	No	21
Previous chemotherapy	Yes	18
	No	18
Discontinuation by adverse events	Yes	3
	No	33
Follow-up time (months)	2 - 26 (median, 16.5)	

PS, performance status; L-SD, long stable disease (for over 6 months); PD, progressive disease

Table 2. Primary antibodies and staining conditions

Antigen	Clone (manufacturer)	Dilution	Retrieval
Rb	4H1, mouse mono (Cell Signaling Technology)	x 800	TRS
pRb	Ser807/811 D20B12, rabbit mono (Cell Signaling Technology)	x 500	TRS
CDK4	DCS-31, mouse mono (Thermo Fisher Scientific)	x 50	TRS
CDK6	EPR4515, rabbit mono (Abcam)	x 100	TRS
cyclin D1	SP4, rabbit mono (Thermo Fisher Scientific)	x 50	citrate buffer
cyclin E1	E-4, mouse mono (Santa Cruz Biotechnology, Inc.)	x 100	no

Rb, retinoblastoma protein; TRS, tris-buffered saline; pRb, phosphorylated Rb; CDK, cyclin-dependent kinase

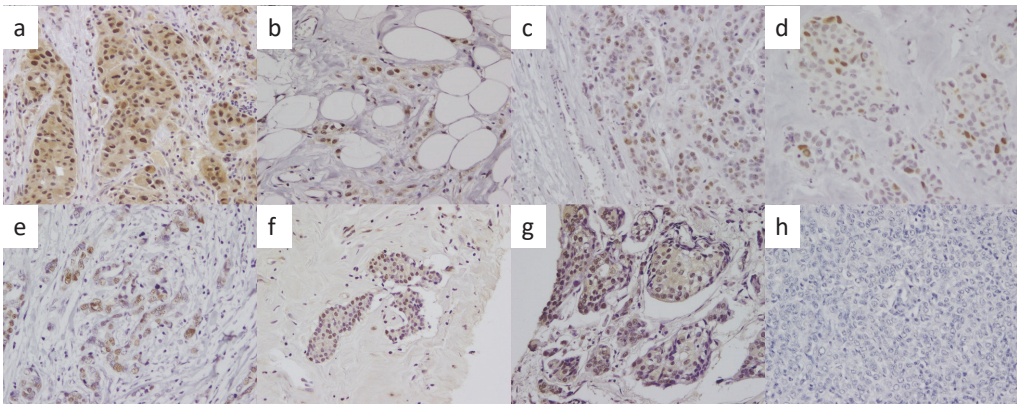

Fig. 1. Representative IHC staining for CDK4 (a), CDK6 (b), Rb (c), pRb (d), cyclin D1 (e), nuclear cyclin E1 (f) and cytoplasmic cyclin E1 (g). Negative control (h).

the HercepTest. Paraffin-embedded tissue sections (4-μm thick) were analyzed using the FISH protocol (Vysis, Downers Grove, IL, USA). An HER2/CEP17 ratio equal to or greater than 2.0 was interpreted as positive for gene amplification.

Regarding exploratory biomarkers, immunostaining was performed using an EnVision Plus kit (Agilent, Santa Clara, CA, USA). Four-μm sections were cut from formalin-fixed paraffin-embedded blocks. After dewaxing and hydration, they were placed in a bath of Target Retrieval Solution, pH 9.0 (Agilent) at 95℃ for 40 min for CDK4 (DCS-31, Thermo Fisher Scientific, Waltham, MA), CDK6 (EPR4515, Abcam, Cambridge, UK), Rb (4H1, Cell Signaling Technology, Danvers, MA) and phospho-Rb (pRb) (Ser807/811) (D20B12, Cell Signaling), or of citrate buffer, pH 6.0 for cyclin D1 (SP4, Thermo Fisher Scientific) at 95℃ for 40 min. The sections were incubated with the primary antibodies overnight at 4℃. The dilutions of primary antibodies were: 1:50 for CDK4 and cyclin D1, 1:100 for CDK6 and cyclin E1 (E-4, Santa Cruz Biotechnology, Inc, Dallas, TX), 1:500 for pRb and 1:800 for Rb. The chromogen used was 3,3' -diaminobenzidine tetrachloride and the sections were counterstained with hematoxylin. The immunohistochemical expression area (%) at any intensity was evaluated. A summarized IHC protocol is shown in Table 2.

Evaluation of IHC data

The cut-off values of the respective biomarkers were defined as the median values of their percentages of positively stained cells. However, as a small percentage of cells was positively stained for CDK6, and nuclear or cytosolic cyclin E1, their cut-off values were defined as 1% of the percentage of positively stained cells. A representative immunostaining image for each biological factor is shown in Fig. 1.

Table 3. Possible predictors for outcomes of patients treated using PAL therapy

Clinical factors	Cut-off values	Biological factors	Cut-off values
Age	50 years or younger/older	ER	1%
PS	0/1 or more	PR	1%
Visceral metastasis	Yes/No	HER2	0/ 1+ or 2+
Liver metastasis	Yes/No	Rb	50%
Bone-only metastasis	Yes/No	pRb	4%
Previous chemotherapy	Yes/No	CDK4	65%
Number of previous endocrine therapies	1 or 2/3 or more	CDK6	1%
Treatment line	1st or 2nd/3rd or later	cyclin D1	50%
L-SD achieved by previous endocrine therapy	Yes/No	nuclear cyclin E1	1%
Combined endocrine therapy	FUL/not	cytosolic cyclin E1	1%
Dose reduction of PAL	Yes/No		

PAL, palbociclib; PS, performance status; L-SD, long stable disease; ER, estrogen receptor; PR, progesterone receptor; HER, human epidermal growth factor receptor; Rb, retinoblastoma protein; pRb, phosphorlated Rb; CDK, cyclin-dependent kinase

Clinical outcome and statistical analysis

Clinical responses to PAL therapy were evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. Changes in serum tumor markers, such as CA 15-3, were used to assist in evaluating clinical responses. Relationships between predictive factors and objective response (OR, complete response [CR] plus partial response [PR]) were assessed by the Fisher's exact test.

PFS was defined as the time from the beginning of PAL therapy to disease progression or death from any cause. The patients who had severe adverse events and discontinued PAL therapy were censored at the time of discontinuation. The PFS rate was estimated according to the Kaplan-Meier method and univariate comparisons between groups were made using the log-rank test.

OS was defined as the time from the beginning of PAL therapy to death from any cause. The OS rate was estimated according to the Kaplan-Meier method and univariate comparisons between groups were made using the log-rank test.

The effects of predictive factors on PFS and OS were quantified in terms of hazard ratios (HRs), estimated and adjusted using the Cox proportional hazards model. All tests were two-tailed and $P < 0.05$ was considered to be significant. Statistical analyses were performed using StatView software (SAS Institute Inc., Tokyo, Japan).

Possible clinical and biological factors influencing the outcomes of patients treated using PAL therapy are listed in Table 3. Only clinically predictive factors demonstrated to be significant are shown in the following Tables.

RESULTS

Predictors for ORR

To explore predictive factors for anti-tumor activity of PAL therapy, clinical and biological factors influencing OR were investigated. Based on the Fisher's exact test, non-visceral metastasis, use of fulvestrant (FUL) and the 1st- or 2nd-line treatment were significant predictors for a better ORR ($P = 0.0080$, $P = 0.0080$ and $P = 0.0080$, respectively). Of note, no OR was observed in patients with PR-negative, CDK6-positive or cytosolic cyclin E1-positive tumors (Table 4).

Predictors for PFS

To explore predictive factors for tumor-controlling activity of PAL therapy, clinical and biological factors influencing PFS were investigated. Based on the log-rank test, non-visceral metastasis and use of FUL were significant predictors for a better PFS ($P = 0.0030$ and $P = 0.0443$, respectively). The 50% PFS and P-values are shown in Table 5. The Kaplan-

Table 4. Predictors for ORR

Possible predictors		ORR	P-value
Non-visceral metastasis	Yes	33.3% (5/15)	0.0080
	No	0% (0/21)	
FUL use	Yes	33.3% (5/15)	0.0080
	No	0% (0/21)	
First- or 2nd-line treatment	Yes	33.3 % (5/15)	0.0080
	No	0% (0/21)	
PR	Positive	17.9% (5/28)	0.5585
	Negative	0% (0/7)	
CDK6	Positive	0% (0/5)	> 0.9999
	Negative	16.1% (5/31)	
cytosolic cyclin E1	Positive	0% (0/9)	0.3017
	Negative	18.5% (5/27)	

ORR, objective response rate; FUL, fulvestrant; PR, progesterone receptor; CDK, cyclin-dependent kinase

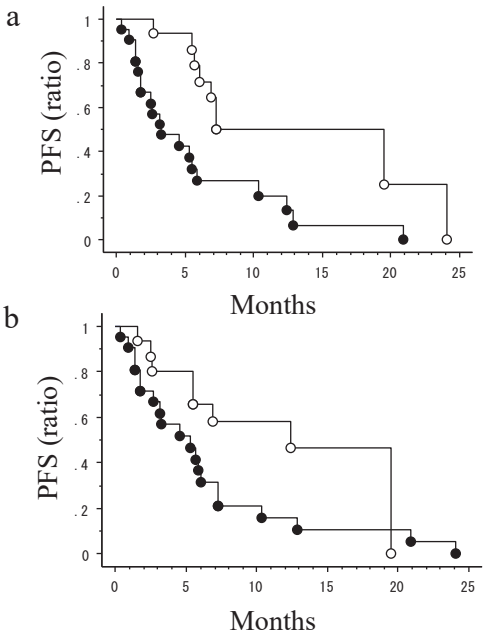

Fig. 2. The Kaplan-Meier curves of PFS in patients treated using PAL therapy stratified by presence (closed circles) or absence (open circles) of visceral metastasis (a) and use (open circles) or non-use (closed circles) of FUL (b).

Meier curves for the two factors are shown in Fig. 2.

According to the Cox proportional hazards model, visceral metastasis (hazard ratio [HR], 4.9; 95% confidence interval [95% CI], 1.8 - 13.3; and P = 0.0019) and PR-negativity (HR, 3.2; 95% CI, 1.0 - 9.5; and P = 0.0411) were independent predictors for a poorer PFS.

Predictors for OS

Clinical and biological factors influencing OS after starting PAL therapy were investigated. Based on the log-rank test, non-visceral metastasis, pRb-negativity and CDK6-negativity were significant predictors for a better OS (P = 0.0281, P = 0.0014 and P = 0.0396). The 50% PFS and P-values are shown in Table 6. The Kaplan-Meier curves for the three factors are shown in Fig. 3.

According to the Cox proportional hazards model, visceral metastasis (HR, 7.2; 95% CI, 1.5 - 33.4; and P = 0.0131) and pRb-positivity (HR, 18.5; 95% CI, 2.3 - 142.9; and P = 0.0060) were independent predictors for a poorer OS.

DISCUSSION

We performed this retrospective study to explore possible predictors for the outcomes of patients with HR-positive, HER2-negative advanced breast cancer treated using the combination of PAL and endocrine therapy. We selected several biological factors related to ER signaling and cell cycle-associated molecules based on the results of recent preclinical and translational studies[2]. The expression levels of these biological factors in primary breast tumors were evaluated by the IHC method. We also analyzed several clinical factors hypothesized to influence the outcomes of the treated patients.

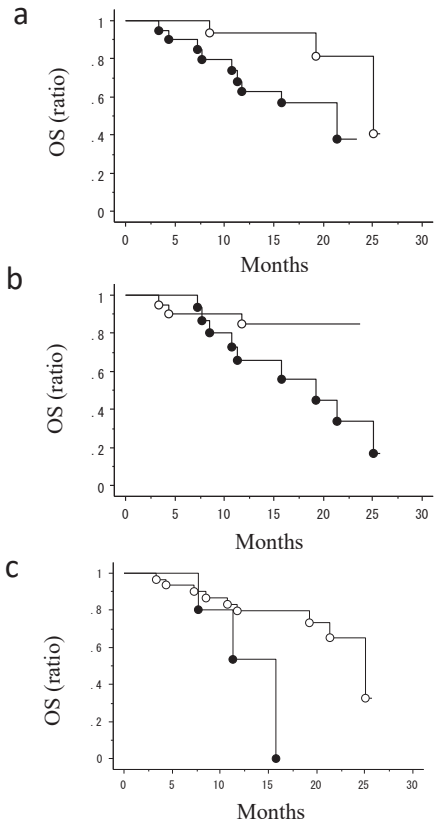

Fig. 3. The Kaplan-Meier curves of OS in patients treated using PAL therapy stratified by the presence (closed circles) or absence (open circles) of visceral metastasis (a), pRb-positive (closed circles) or -negative (open circles) (b) and CDK6-positive (closed circles) or -negative (open circles) (c).

Table 5. Predictors for PFS

Possible predictors		50% PFS	P-value
Non-visceral metastasis	Yes	18.2	0.0030
	No	3.2	
FUL use	Yes	12.1	0.0443
	No	5.0	
PR	Positive	6.4	0.4977
	Negative	4.0	
CDK6	Positive	2.6	0.1404
	Negative	6.1	

PFS, progression-free survival; FUL, fulvestrant; PR, progesterone receptor; CDK, cyclin-dependent kinase

Table 6. Predictors for OS

Possible predictors		50% OS	P-value
Non-visceral metastasis	Yes	25.0	0.0281
	No	21.3	
pRb	Positive	15.6	0.0014
	Negative	NR	
CDK6	Positive	12.6	0.0396
	Negative	24.8	

OS, overall survival; pRb, phosphorylated retinoblstoma protein; NR: not reached; CDK, cyclin-dependent kinase

This study revealed several factors related to the responsiveness and the resistance to PAL-containing therapy.

Regarding the clinically predictive factors, non-visceral metastasis, FUL as a combination partner and 1st- or 2nd-line use for the treatment of advanced breast cancer were significant predictors for better outcomes of patients treated using PAL therapy. It is well known that visceral metastasis is unlikely to respond to endocrine therapy compared with non-visceral metastasis. Therefore, the combination of endocrine therapy and PAL may be more efficacious for non-visceral metastasis than visceral metastasis. Indeed, non-visceral metastasis was a significant predictor for the ORR, PFS and OS in this study (Tables 4-6, Figs. 2, 3). The combined use of FUL and PAL was suggested to be more potent than that of an aromatase inhibitor and PAL (Table 4). As the median number of previous endocrine therapies before PAL therapy was three, most patients in this study previously received different types of endocrine therapy (Table 1). Thus, the combined use of FUL being more efficacious than that of an aromatase inhibitor may be reasonable. In addition, it is well known that 1st-line use of antitumor agents is more effective than their later use. It may therefore be reasonable that the 1st- or 2nd-line use of PAL therapy was more efficacious than the 3rd- or later line use (Table 4).

The multivariate analysis revealed that PR-negativity in primary breast tumors was an independent predictor for a poorer PFS in this study. A translational study using samples obtained from a large prospective randomized study, POLOMA-3, demonstrated that a lower expression level of PR correlated with a poor response to combined PAL

and FUL therapy[4]. PR-negativity in ER-positive breast tumors is an important predictor for a poorer response to endocrine therapy. PR expression may be also a predictor for response to combined PAL and endocrine therapy.

Regarding the exploration of biological factors for predicting patient outcomes, Rb loss or dysfunction was reported to cause *de novo* or acquired resistance to CDK4/6 inhibitors[2]. However, Rb negativity was not a significant predictor for the ORR, PFS or OS in this study. In contrast, pRb positivity was an independent predictor for OS (Table 6). It is well known that pRb is an activated form of Rb mediated by the cyclin D/CDK4/6/Rb/E2F pathway that regulates the G1-S transition in the cell cycle. Lower expression levels of Rb were reported to correlate with a shorter PFS of patients with metastatic breast tumors treated using PAL-containing therapy in one translational study[8], but not in another study[7]. There is no published report on the relationship between basal pRb expression levels in breast tumors and outcomes of patients treated using a CDK4/6 inhibitor and endocrine therapy. Rb activation by other growth-promoting pathways, such as growth factor receptor pathways, in breast tumors may induce malignant progression and deteriorate the patient outcome.

The expression levels of CDK4 and CDK6 were hypothesized to correlate with responses to CDK4/6 inhibitor-containing therapy. Although CDK4 expression in primary breast tumors was not correlated with the outcomes of patients treated using PAL therapy, CDK6 expression was correlated with the ORR, PFS and OS in this study (Tables 4 - 6, Fig. 3). In preclinical models, gene amplification or overexpression of CDK6 was noted after the acquisition of resistance to CDK4/6 inhibitors in ER-positive, HER2-negative breast cancer cell lines[9, 10]. This suggests that CDK6 overexpression plays an important role in the development of resistance to CDK4/6 inhibitors. In contrast, as previously reported by a translational study[11], cyclin D1 expression was not correlated with the outcomes of the patients in this study.

Although CDK4/6 plays an essential role in the phosphorylation of Rb and cell cycle transition from the G1 to S phase, the cyclin E1/CDK2 pathway is also known to phosphorylate Rb and promote G1-S transition[2]. Abnormal activation of the cyclin E1/CDK2 pathway was previously suggested to promote resistance to CDK4/6 inhibitors in preclinical studies[2, 8]. In particular, overexpression of cytosolic cyclin E1 was reported to be a candidate factor for the resistance to CDK4/6 inhibitors[8]. No OR was achieved by PAL therapy in patients with primary breast tumors expressing cytosolic cyclin E1 (Table 4), but cytosolic cyclin E1 expression was not correlated with the PFS or OS in this study.

There are some limitations in this study. The number of study subjects was small, the outcomes of the patients were not precisely evaluated because of the retrospective observation, the expression levels of biological factors were examined in primary breast tumors, but not in metastatic or recurrent lesions, and the evaluation of their expression levels was not standardized. However, this study suggested that biological factors, such as PR, pRb, CDK6 and cytosolic cyclin E1, in primary tumors can predict the outcomes of patients with ER-positive, HER2-negative advanced breast cancer treated using PAL and endocrine therapy. These biological factors may be useful to select advanced breast cancer patients who should be treated or not with PAL and endocrine therapy.

ACKNOWLEDGMENTS

We thank Mrs. Kaoru Tsuboi and Megumi Kuriyama for their technical assistance. YY, JK and NK carried out and designed the experiments, and drafted the manuscript. RO aided in conception and execution of the data collection. EK and TM made substantial contributions to the design of the

experiments and reviewed the manuscript critically for important intellectual content. All authors read and approved the final manuscript, and agree to be accountable for the integrity of the work.

FUNDING

This study was partially supported by Research Project Grants from Kawasaki Medical School and MEXT/JSPS KAKENHI Grant Number JP17K10566.

CONFLICTS OF INTEREST

JK received advisory/consultation fees and research funding from Takeda Pharmaceutical Co. JK also received research funding from Eisai Co. The other authors declare that they have no conflicts of interest.

ETHICAL APPROVAL

The protocol of the present study was approved by the ethics committee of Kawasaki Medical School and Hospital (approval number: 3139).

REFERENCES

1）Spring LM, Wander SA, Andre F, Moy B, Turner NC, Bardia A: Cyclin-dependent kinase 4 and 6 inhibitors for hormone receptor-positive breast cancer: past, present, and future. Lancet. 2020; 395: 817-827. doi: 10.1016/S0140-6736(20)30165-3.

2）Pandey K, An HJ, Kim SK, Lee SA, Kim S, Lim SM, Kim GM, Sohn J, Moon YW: Molecular mechanisms of resistance to CDK4/6 inhibitors in breast cancer: A review. Int J Cancer. 2019; 145: 1179-1188. doi: 10.1002/ijc.32020.

3）O'Leary B, Cutts RJ, Liu Y, *et al.* : The Genetic Landscape and Clonal Evolution of Breast Cancer Resistance to Palbociclib plus Fulvestrant in the PALOMA-3 Trial. Cancer Discov. 2018; 8: 1390-1403. doi: 10.1158/2159-8290.CD-18-0264.

4）Cristofanilli M, DeMichele A, Giorgetti C, *et al.*: Predictors of prolonged benefit from palbociclib plus fulvestrant in women with endocrine-resistant hormone receptor-positive/human epidermal growth factor receptor 2-negative metastatic breast cancer in PALOMA-3. Eur J Cancer. 2018; 104: 21-31. doi: 10.1016/j.ejca.2018.08.011.

5）Turner NC, Liu Y, Zhu Z, *et al.*: Cyclin E1 Expression and Palbociclib Efficacy in Previously Treated Hormone Receptor-Positive Metastatic Breast Cancer. J Clin Oncol. 2019; 37: 1169-1178. doi: 10.1200/JCO.18.00925.

6）Formisano L, Lu Y, Servetto A, *et al.*: Aberrant FGFR signaling mediates resistance to CDK4/6 inhibitors in ER+ breast cancer. Nat Commun. 2019; 10: 1373. doi: 10.1038/s41467-019-09068-2.

7）Finn RS, Liu Y, Zhu Z, *et al.*: Biomarker Analyses of Response to Cyclin-Dependent Kinase 4/6 Inhibition and Endocrine Therapy in Women with Treatment-Naïve Metastatic Breast Cancer. Clin Cancer Res. 2020; 26: 110-121. doi: 10.1158/1078-0432.CCR-19-0751.

8）Vijayaraghavan S, Karakas C, Doostan I, *et al.*: CDK4/6 and autophagy inhibitors synergistically induce senescence in Rb positive cytoplasmic cyclin E negative cancers. Nat Commun. 2017; 8: 15916. doi: 10.1038/ncomms15916.

9）Yang C, Li Z, Bhatt T, Dickler M, Giri D, Scaltriti M, Baselga J, Rosen N, Chandarlapaty S: Acquired CDK6 amplification promotes breast cancer resistance to CDK4/6 inhibitors and loss of ER signaling and dependence. Oncogene. 2017; 36: 2255-2264. doi: 10.1038/onc.2016.379.

10）Iida M, Nakamura M, Tokuda E, Toyosawa D, Niwa T, Ohuchi N, Ishida T, Hayashi SI: The p21 levels have the potential to be a monitoring marker for ribociclib in breast cancer. Oncotarget. 2019; 10:4907-4918. doi: 10.18632/oncotarget.27127.

11）Finn RS, Crown JP, Lang I, *et al.*: The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncol. 2015; 16: 25-35. doi: 10.1016/S1470-2045(14)71159-3.