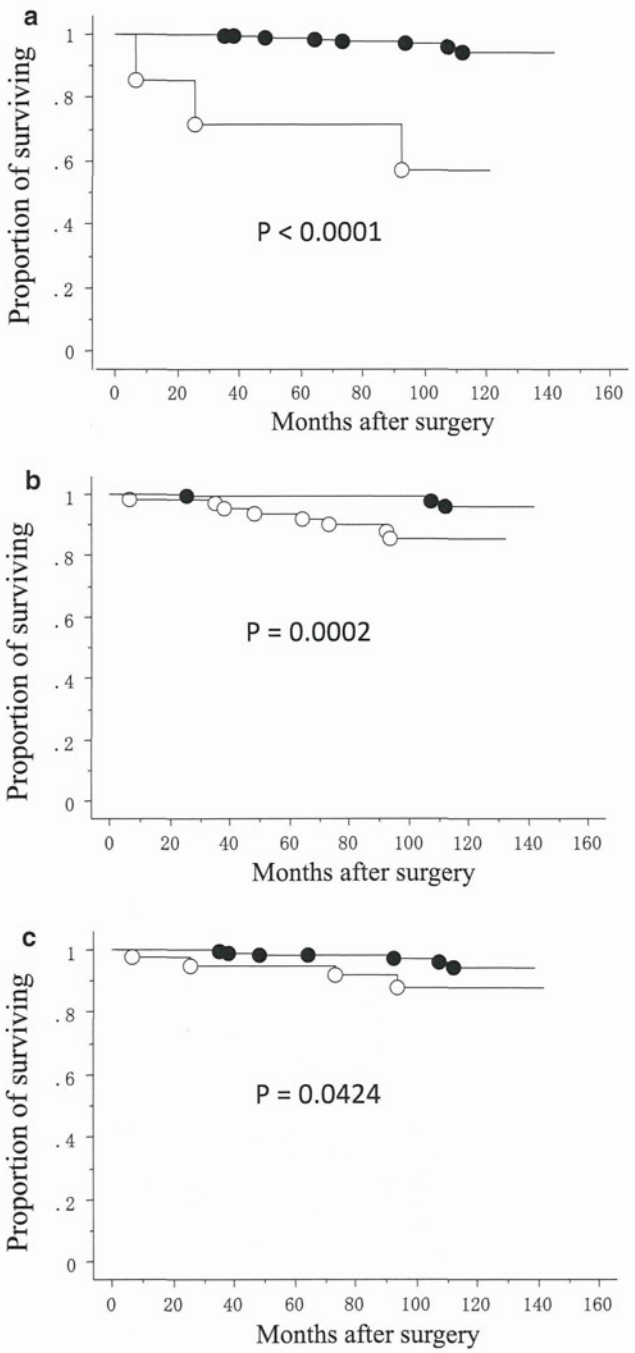

Fig. 4 DRFS according to LVI (**a**), PgR status (**b**), and Ki67 LI (**c**). **a** *Open circles*, marked LVI, *filled circles* without marked LVI. **b** *Open circles* PgR-negative tumors, *filled circles* PgR-positive tumors. **c** *Open circles* tumors showing high Ki67 LI, *filled circles* tumors not showing high Ki67 LI

Results

Clinicopathological characteristics of the study subjects

The median patient age was 59 years; 59 % received breast-conserving surgery; the mean tumor size was 1.9 cm; and 80 % were node negative. All patients had ER-positive breast tumors. Tamoxifen alone was given to 170 (65 %) of the patients (Table 1). At the median follow-up period of 98 months, 11 patients had distant recurrence (six in bone, two in bone and lung, two in liver, and one in lung). At the median follow-up period of 99 months, four patients had died of breast cancer.

The distribution of the percentages of hormone receptor-positive cells in each tumor sample is shown in Fig. 1. The percentages of ER-positive cells were over 90 % in most cases (Fig. 1a), but those of PgR-positive cells were widely distributed from 0 to 100 % (Fig. 1b). These findings indicate that the majority of tumors were strongly positive for ER.

The distribution of Ki67 LI is shown in Fig. 2. The median Ki67 LI was 17.6 % (range 0.8–67.0 %). The Ki67 LI correlated well with the histological grade. The average Ki67 LIs were 14.7 % for grade I, 19.1 % for grade II, and 27.0 % for grade III. When the cutoff values of Ki67 LI were changed, the value of 30 % discriminated patients with poor or good prognosis; therefore, the cutoff value of Ki67 LI was defined as 30 % in this study (Table 2).

For HER1, any completely membranous staining was considered positive, regardless of its intensity or proportion [9]. HER1 expression was observed in only 8.4 % of cases (22 out of 261, Table 2). The mean Ki67 LI was significantly higher in HER1-positive tumors than in HER1-negative tumors (25.7 versus 18.2 %, $P = 0.0012$).

For IGF-1R, cytoplasmic staining was scored by the Histoscore system, multiplying the products of the percentage of cells stained at a given staining intensity (0–100) by the staining intensity score (0, none; 1, weak; 2, moderate; 3, intense): 0–10 points were considered as negative, 11–100 points as weak, 101–200 points as moderate, and 201–300 points as strongly positive [10]. The IGF-1R expression was categorized as negative in 64 cases (24.5 %), weak in 171 cases (65.5 %), and strong in 26 cases (10.0 %) (Table 2). No significant correlation among IGF-1R expression and other clinicopathological factors was observed. IGF-1R expression in tumor cells was not a significant prognostic factor for either DRFS or BCSS.

For ALDH1, tumor cells with cytoplasmic staining were considered to be ALDH1-positive cells [11]. The proportions of ALDH1-positive cells and the number of cases were as follows: <10 % in 111 cases (42.5 %), 10–50 % in 115 cases (44.1 %), and >50 % in 34 cases (13.0 %, Table 2). No significant correlation between ALDH1 expression and other clinicopathological factors was observed. ALDH1 expression in tumor cells was not a significant prognostic factor for either DRFS or BCSS.

Representative findings of IHC for Ki67, HER1, IGF-1R, and ALDH1 are shown in Fig. 3.

Table 3 Analyses of distant relapse-free survival

Variable	Category	Number	Univariate analysis	Multivariate analysis		
			P value	Hazard ratio	95 % CI	*P* value
LVI	Marked	7	<0.0001	21.8	5.5–86.2	<0.0001
	Not	250				
PgR	Negative	69	0.0002	10.3	2.7–39.8	0.0007
	Positive	192				
Ki67 LI	≥30 %	40	0.0424	NA	NA	NA
	<30 %	219				
Grade	III	41	0.0071	NA	NA	NA
	I/II	220				

NA not applicable

Univariate analyses of DRFS and BCSS

Marked LVI (Fig. 4a, $P < 0.0001$), PgR negativity (Fig. 4b, $P = 0.0002$), high Ki67 LI (Fig. 4c, $P = 0.0424$), and histological grade III ($P = 0.0071$) were significant prognostic factors for DRFS by the log-rank test (Table 3). In contrast, other factors, such as tumor size, age, nodal status, HER2 status, HER1 status, ALDH1 status, and IGF-1R status, were not significant.

Marked LVI (Fig. 5a, $P < 0.0001$), high Ki67 LI (Fig. 5b, $P = 0.0009$), PgR negativity (Fig. 5c, $P = 0.0205$), and histological grade III ($P = 0.0021$) were significant prognostic factors for BCSS by the log-rank test (Table 4). In contrast, other factors such as age, tumor size, nodal status, HER2 status, HER1 status, ALDH1 status, and IGF-1R status were not significant.

Multivariate analyses of distant metastasis and breast cancer-specific death

Cox proportional hazards model revealed that marked LVI [hazard ratio (HR) 21.8, 95 % confidence interval (CI) 5.5–86.2, $P < 0.0001$] and PgR negativity (HR 10.3, 95 % CI 2.7–39.8, $P = 0.0007$) were independently worse prognostic factors for distant metastasis (Table 3). The Cox proportional hazards model revealed that marked LVI (HR 287.3, 95 % CI 5.6–14817.5, $P = 0.0049$), high Ki67 LI (HR 19.6, 95 % CI 1.4–250.0, $P = 0.0292$), and PgR negativity (HR 25.1, 95 % CI 1.2–536.5, $P = 0.0389$) were independently worse prognostic factors for breast cancer-specific death (Table 4).

Discussion

Whether chemotherapy should be postoperatively administered in addition to endocrine therapy to patients with ER-positive and/or PgR-positive, HER2-negative breast cancer remains unanswered. In daily practice, physicians always take into account two important issues: the risk of recurrence, and the biological characteristics responsible for response to certain therapies. The former includes clinicopathological factors such as nodal status, tumor size, histological grade, LVI, and others. The latter includes biomarkers such as ER, PgR, HER2, Ki67 LI, and others; however, it is difficult for physicians to distinguish which factors are more important than others [1–3]. To clarify the significance of these factors and also to explore new prognostic factors, we conducted this multi-institute cohort study.

To exclude the effect of adjuvant chemotherapy on patients' prognosis, breast cancer patients postoperatively treated with endocrine therapy alone were recruited to this study. As expected, the majority of patients had ER-positive, HER2-negative breast cancer at earlier stages. They received standard adjuvant endocrine therapies, tamoxifen alone, aromatase inhibitor alone, or tamoxifen followed by aromatase inhibitor in cases of postmenopausal status or tamoxifen alone, or luteinizing hormone-releasing hormone (LH–RH) agonist plus tamoxifen or LH–RH agonist alone in cases of premenopausal status (Table 1). Although they were recruited from three different hospitals, the clinicopathological characteristics and adjuvant endocrine therapy used were not significantly different (data not shown). This is probably because most physicians working for the respective hospitals basically followed the recommendations for the selection of postoperative adjuvant treatment for early breast cancer provided by the St. Gallen Consensus Conference. Additionally, there was no significant difference in DRFS or BCSS among the types of endocrine therapy (data not shown).

To focus on the occurrence of fatal recurrence and exclude the influence of local therapies on overall recurrence rates, DRFS and BCSS rather than disease-free survival (DFS) and overall survival were investigated in this study. It was found that, because there were some

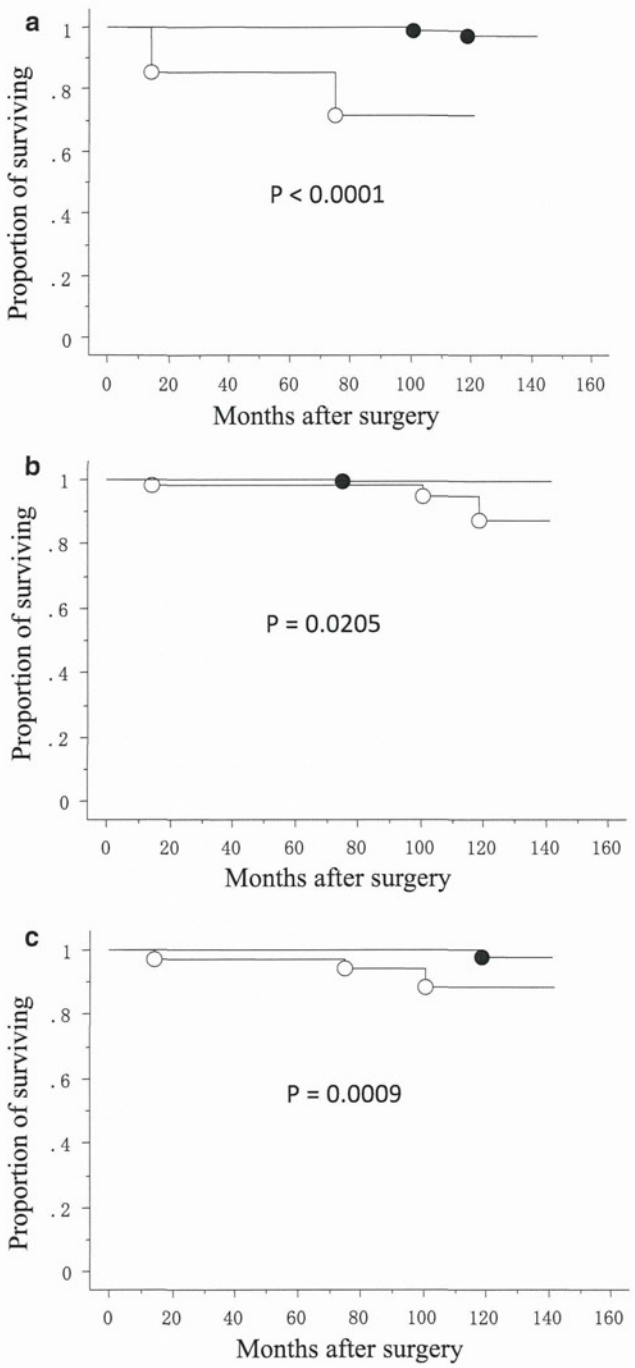

Fig. 5 BCSS according to LVI (**a**), PgR status (**b**), and Ki67 LI (**c**). **a** *Open circles* marked LVI, *filled circles* without marked LVI. **b** *Open circles* PgR-negative tumors, *filled circles* PgR-positive tumors. **c** *Open circles* tumors showing high Ki67 LI, *filled circles* tumors not showing high Ki67 LI

differences in the indication for breast-conserving surgery among the three hospitals, local recurrence rates significantly differed among them (data not shown).

Multivariate analyses of both distant metastasis and breast cancer-specific death showed that three pathological biomarkers, marked LVI, PgR negativity, and high Ki67 LI, were independently worse prognostic factors in this study population (Tables 3, 4). It is likely that, because of the small proportion of cases with large and/or node-positive tumors, the nodal status and tumor size became nonindependent prognostic factors in this study (Table 1). It should be noted that histological grade was not selected as an independent prognostic factor. Histological grade correlated well with Ki67 LI in this study population. When removing Ki67 LI from prognostic variables, histological grade became an independent prognostic factor (data not shown). These findings indicate that histological grade and Ki67 LI influenced each other in terms of prognostic variables and Ki67 LI may have removed histological grade from significant prognostic factors in this study.

Lymphovascular invasion has been associated with poor outcome in patients with breast cancer, in particular, node-negative and/or small tumors [12]. Very recently, a large cohort study reported that LVI provided a strong predictor of outcome in patients with invasive breast cancer and should be incorporated into breast cancer staging systems [13]. The results of this study totally support these findings; however, some points should be clarified before the routine use of LVI as a prognostic factor. First, how to objectively evaluate LVI in breast tumors remains to be resolved. The use of IHC for detecting an endothelial-specific marker such as D2-40 and quantitative imaging techniques may contribute to the prognostic significance of LVI. In addition, the significance of LVI grading is still unclear. In this study, study subjects were divided into two groups according to the grade of LVI, marked LVI or not [6]. This categorization successfully predicted the risk of distant recurrence and breast cancer-specific death. In contrast, when using other categorizations such as absence and mild versus moderate and marked, LVI became a nonindependent prognostic factor.

It has been indicated that PgR status is an independent predictive factor of benefit from adjuvant endocrine therapy and that PgR status should be taken into account when discussing risk reductions expected from endocrine therapy with individual breast cancer patients [14]. In addition, it was hypothesized that loss of PgR in ER-positive breast cancer is a surrogate marker for increased activity of growth factor receptor tyrosine kinases, such as HER1 and HER2, that cause lower PgR expression and tamoxifen resistance in some patients [15].

The significance of HER1 expression in tumor cells remains controversial in terms of prognostic power in breast cancer patients [16]. Recent studies have suggested that HER1 overexpression correlated with worse outcome in patients with breast cancer of the triple-negative phenotype [17–20]. HER1 expression was not an independent prognostic factor in this study population, which consisted

Table 4 Analyses of breast cancer-specific survival

Variable	Category	Number	Univariate analysis	Multivariate analysis		
			P value	Hazard ratio	95 % CI	*P* value
LVI	Marked	7	<0.0001	287.3	5.6–14817.5	0.0049
	Not	250				
Ki67 LI	≥30 %	40	0.0009	19.6	1.4–250.0	0.0292
	<30 %	219				
PgR	Negative	69	0.0205	25.1	1.2–536.5	0.0389
	Positive	192				
Grade	III	41	0.0021	NA	NA	NA
	I/II	220				

NA not applicable

of patients with ER-positive, HER2-negative breast cancer. Otherwise, the proportion of HER1-expressing tumors was very small but these tumors showed higher Ki67 LI in this study as described in the "Results." Further studies are clearly needed to develop a quantitative assay procedure for HER1 expression and to elucidate an appropriate cutoff point of HER1 expression as a prognostic marker in breast cancer patients.

It has been indicated that the IGF1-R signaling pathway plays important roles in cell survival and resistance to endocrine therapy and anti-HER2 therapy through the PI3K/AKT/mTOR cascade [21–23]; however, the clinical significance of IGF1-R expression in tumor cells remains controversial. It is well known that the expression level of IGF1-R is not the sole mediator to regulate activity of the PI3K/AKT/mTOR signaling pathway. In addition, there are confounding factors influencing IGF signaling, such as the activation mechanism of IGF1-R and various IGF binding proteins [24].

The cancer stem cell theory in solid tumors including breast cancer has been widely accepted as a key paradigm to develop new anticancer strategies [4]. Because breast cancer stem cells may play an important role in developing resistance to endocrine therapy, expression levels of a putative cancer stem cell marker, ALDH1 [25], in tumor cells were investigated in this study. Unexpectedly, the proportion of ALDH1-positive tumor cells was not a prognostic factor in this study. Recent studies have suggested that ALDH1 positivity may have prognostic value in patients with triple-negative breast cancer [26]. It has been speculated that suitable cancer stem cell markers are different among breast cancer subtypes [27, 28]. It seems that ALDH1-positive tumor cells were not cancer stem cells in the ER-positive, HER2-negative breast tumors investigated in this study. Other cancer stem cell markers such as cell surface markers CD44 and CD24 might be more useful in studying the significance of the proportion of cancer stem cells as a prognostic variable in ER-positive, HER2-negative breast cancer.

In conclusion, this retrospective multi-institute cohort study indicated that three pathological factors, marked LVI, PgR negativity, and high Ki67 LI, were independently worse prognostic factors in early breast cancer patients treated with endocrine therapy alone. These factors were representative biomarkers for tumor invasiveness, endocrine responsiveness, and tumor cell proliferation. Although it is uncertain whether additional chemotherapy is beneficial or not for patients with these worse prognostic factors, because these factors are relatively easy to identify in daily practice, physicians should take into account these prognostic factors for the choice of chemotherapy additional to endocrine therapy in patients with ER-positive, HER2-negative breast cancer.

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References

1. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Panel members. Strategies for subtypes—dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol. 2011;22:1736–47.
2. Lim E, Winer EP. Adjuvant chemotherapy in luminal breast cancers. Breast. 2011;20(Suppl 3):S128–31.
3. Goldhirsch A, Ingle JN, Gelber RD, Coates AS, Thürlimann B, Panel members. Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2009. Ann Oncol. 2009;20:1319–29.
4. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of

American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol. 2010;28:2784–95.
5. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology. 1991;19:403–10.
6. Ito M, Moriya T, Ishida T, Usami S, Kasajima A, Sasano H, Ohuchi N. Significance of pathological evaluation for lymphatic vessel invasion in invasive breast cancer. Breast Cancer. 2007;14(4):381–7.
7. Moriya T, Sakamoto K, Sasano H, Kawanaka M, Sonoo H, Manabe T, Ito J. Immunohistochemical analysis of Ki-67, p53, p21, and p27 in benign and malignant apocrine lesions of the breast: its correlation to histologic findings in 43 cases. Mod Pathol. 2000;13:13–8.
8. Hudis CA, Barlow WE, Costantino JP, Gray RJ, Pritchard KI, Chapman JA, et al. Proposal for standardized definitions for efficacy end points in adjuvant breast cancer trials: the STEEP system. J Clin Oncol. 2007;25:2127–32.
9. Cabioglu N, Gong Y, Islam R, Broglio KR, Sneige N, Sahin A, et al. Expression of growth factor and chemokine receptors: new insights in the biology of inflammatory breast cancer. Ann Oncol. 2007;18:1021–9.
10. Fu P, Ibusuki M, Yamamoto Y, Hayashi M, Murakami K, Zheng S, Iwase H. Insulin-like growth factor-1 receptor gene expression is associated with survival in breast cancer: a comprehensive analysis of gene copy number, mRNA and protein expression. Breast Cancer Res Treat. 2011;130:307–17.
11. Ohi Y, Umekita Y, Yoshioka T, Souda M, Rai Y, Sagara Y, et al. Aldehyde dehydrogenase 1 expression predicts poor prognosis in triple-negative breast cancer. Histopathology. 2011;59:776–80.
12. Hanrahan EO, Valero V, Gonzalez-Angulo AM, Hortobagyi GN. Prognosis and management of patients with node-negative invasive breast carcinoma that is 1 cm or smaller in size (stage 1; T1a, bN0M0): a review of the literature. J Clin Oncol. 2006;24:2113–22.
13. Mohammed RA, Martin SG, Mahmmod AM, Macmillan RD, Green AR, et al. Objective assessment of lymphatic and blood vascular invasion in lymph node-negative breast carcinoma: findings from a large case series with long-term follow-up. J Pathol. 2011;223:358–65.
14. Bardou VJ, Arpino G, Elledge RM, Osborne CK, Clark GM. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. J Clin Oncol. 2003;21:1973–9.
15. Arpino G, Weiss H, Lee AV, Schiff R, De Placido S, Osborne CK, Elledge RM. Estrogen receptor-positive, progesterone receptor-negative breast cancer: association with growth factor receptor expression and tamoxifen resistance. J Natl Cancer Inst. 2005;97:1254–61.
16. Rampaul RS, Pinder SE, Nicholson RI, Gullick WJ, Robertson JF, Ellis IO. Clinical value of epidermal growth factor receptor expression in primary breast cancer. Adv Anat Pathol. 2005;12:271–3.
17. Kurebayashi J. Possible treatment strategies for triple-negative breast cancer on the basis of molecular characteristics. Breast Cancer. 2009;16:275–80.
18. Rakha EA, El-Sayed ME, Green AR, Lee AH, Robertson JF, Ellis IO. Prognostic markers in triple-negative breast cancer. Cancer. 2007;109:25–32.
19. Cheang MC, Voduc D, Bajdik C, Leung S, McKinney S, Chia SK, et al. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. Clin Cancer Res. 2008;14:1368–76.
20. Viale G, Rotmensz N, Maisonneuve P, Bottiglieri L, Montagna E, Luini A, et al. Invasive ductal carcinoma of the breast with the "triple-negative" phenotype: prognostic implications of EGFR immunoreactivity. Breast Cancer Res Treat. 2009;116:317–28.
21. Ellis MJ, Jenkins S, Hanfelt J, Redington ME, Taylor M, Leek R, et al. Insulin-like growth factors in human breast cancer. Breast Cancer Res Treat. 1998;52:175–84.
22. Nahta R, Yu D, Hung MC, Hortobagyi GN, Esteva FJ. Mechanisms of disease: understanding resistance to HER2-targeted therapy in human breast cancer. Nat Clin Pract Oncol. 2006;3:269–80.
23. Fox EM, Miller TW, Balko JM, Kuba MG, Sánchez V, Smith RA, et al. A kinome-wide screen identifies the insulin/IGF-I receptor pathway as a mechanism of escape from hormone dependence in breast cancer. Cancer Res. 2011;71:6773–84.
24. Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer. 2008;8:915–28.
25. Liu S, Wicha MS. Targeting breast cancer stem cells. J Clin Oncol. 2010;28:4006–12.
26. Douville J, Beaulieu R, Balicki D. ALDH1 as a functional marker of cancer stem and progenitor cells. Stem Cells Dev. 2009;18:17–25.
27. Park SY, Lee HE, Li H, Shipitsin M, Gelman R, Polyak K. Heterogeneity for stem cell-related markers according to tumor subtype and histologic stage in breast cancer. Clin Cancer Res. 2010;16:876–87.
28. Ricardo S, Vieira AF, Gerhard R, Leitão D, Pinto R, Cameselle-Teijeiro JF, et al. Breast cancer stem cell markers CD44, CD24 and ALDH1: expression distribution within intrinsic molecular subtype. J Clin Pathol. 2011;64:937–46.

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ORIGINAL ARTICLE

Paclitaxel-induced peripheral neuropathy in patients receiving adjuvant chemotherapy for breast cancer

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Abstract
Background The long-term outcomes and risk factors of paclitaxel-induced peripheral neuropathy (PIPN) have not yet been fully elucidated.
Methods We identified 219 breast cancer patients who received paclitaxel as adjuvant chemotherapy between 2002 and 2009. We retrospectively analyzed the incidence, time to onset, duration, and risk factors for PIPN by chart review.
Results Of the 219 patients, 212 developed PIPN (97%) during a median follow-up time of 57 months (range 5.3–95.5). Median time to PIPN onset was 21 days (range 11–101) for the entire patient population: 35 days (range 14–77) for weekly administration and 21 days (range 11–101) for tri-weekly administration. PIPN caused termination of paclitaxel treatment in 7 patients (4%). Median duration of PIPN was 727 days (range 14–2621 days). PIPN persisted in 64 and 41% of patients at 1 and 3 years after initiating paclitaxel, respectively. Age ≥60 years and severity of PIPN were significantly associated with PIPN duration.
Conclusions PIPN persists longer in older patients and in those who experience severe neuropathy. Further studies to identify the risk factors for PIPN are warranted.

Keywords Breast cancer · Paclitaxel · Peripheral neuropathy

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Introduction

Paclitaxel (PTX) is a key component of many therapeutic regimens in both early-stage and metastatic breast cancer [1–4]. PTX, a microtubule-stabilizing agent, binds to microtubules and abolishes their dynamic behavior, leading to inhibition of cell proliferation [5]. The agent is known to cause peripheral neurotoxicity (PN), which may result in discontinuation of treatment and poor quality of life.

The incidence of PTX-induced PN (PIPN) is known to depend on several factors, including dosages per cycle, treatment schedule, duration of infusion, cumulative dosage, and co-morbidity such as diabetes [6–11]. Although the clinical response of tumors to PTX is an important factor in selecting a chemotherapy regimen, it is also prudent to evaluate the risk of developing PN associated with each regimen, especially for patients already at high risk for neuropathy. The risk of sensory neuropathy is proportional to the dose of PTX administered. Grade 3 or 4 sensory neurotoxicity occurs in 20–35% of patients receiving 250 mg/m^2 every 3 weeks compared to 5–12% using doses ≤200 mg/m^2 every 3 weeks [12]. The weekly schedule is associated with higher neurotoxicity than the tri-weekly schedule. In a previous study, grade 3 neuropathy occurred significantly more often with the weekly regimen than with the tri-weekly regimen (24 vs. 12%) [13]. In another study, which compared weekly versus

tri-weekly PTX dosages, it was reported that grade 2, 3, or 4 neuropathy occurred more frequently with weekly than with tri-weekly PTX administration (27 vs. 20%, respectively) [14].

The time to onset of PIPN was previously determined in a phase III trial of patients with metastatic breast cancer treated with PTX (175 mg/m^2) every 3 weeks; the mean total dose at the onset of grade 2 neurotoxicity was 715 mg/m^2 [15]. However, there are limited data available describing the outcome of PIPN and risk factors of severe PN. We therefore conducted a retrospective study to determine the duration of PIPN and to identify potential factors predicting severe or persistent PN.

Patients and methods

Data collection

This study included breast cancer patients treated with PTX as adjuvant chemotherapy at the National Cancer Center Hospital between 2002 and 2009. All patients met the following criteria: female gender; age >18 years; recipients of lumpectomy or mastectomy; and presentation of more than one axillary lymph node metastasis, as determined pathologically. The following patients were excluded from this study: those previously treated with PTX, those who presented with severe neuropathy before initiating PTX treatment, and those who discontinued PTX treatment after only 1 cycle for any reason.

We performed chart reviews for all patients to obtain the following information: age; gender; stage; hormonal status; human epidermal growth factor receptor-2 (HER2) status; previous surgical procedures (lumpectomy or mastectomy); adjuvant chemotherapy; adjuvant radiotherapy; PTX administration schedule; date of the first documentation of PIPN; maximum grade of PIPN; date of disappearance of PIPN symptoms. This study was approved by the local institutional review board.

Treatment schedule

Chemotherapy consisted of anthracycline followed by PTX regimens as generally recommended for high-risk breast cancer patients, according to the St. Gallen risk criteria at our division [16, 17]. However, therapeutic options could vary based on the physician's discretion. Patients received either 80 mg/m^2 of PTX on days 1, 8, and 15 of each 21-day interval for 4 cycles, following anthracycline plus cyclophosphamide (AC) (weekly administration schedule), or 175 mg/m^2 of PTX on day 1 of each 21-day interval for 4 cycles, following AC (tri-weekly administration schedule).

Grading of PIPN

Patients were evaluated during and after chemotherapy by medical oncologists. We graded PIPN retrospectively according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0 [18]. Grade 1 PIPN had paresthesias including tingling, but not interfering with function, while grade 2 had sensory alterations or paresthesias interfering with function but not interfering with activities of daily living (ADL). Grade 3 had sensory alterations or paresthesias interfering with ADL. Patients were determined to have PIPN if their score for sensory neuropathy was grade 1 or higher. The severity of pain was not evaluated in this study because of insufficient data.

Statistical analysis

The time to onset of PIPN was defined as the time from the date of PTX administration to the date of the first documentation of PIPN. The duration of PIPN was defined as the time from the date of first documentation of PIPN to the date of disappearance of the PIPN symptoms described. The time to onset and duration of PIPN were estimated by the Kaplan–Meier method. We used multivariate Cox regression analysis to identify the variables associated with the time to onset and duration of PIPN. Furthermore, to identify the risk factors for PIPN above grade 2, we applied multivariate logistic regression analysis. A 2-sided $P < 0.05$ was considered statistically significant. All analyses were performed by SAS software, version 9.2 (SAS Institute, Cary, NC, USA).

Results

Patient characteristics

Of the 227 patients initially identified, 2 were excluded due to severe neuropathy induced by combination chemotherapy with AC before being treated with PTX. Several patients discontinued systemic therapy before completion of 1 cycle due to the following adverse events: severe liver dysfunction (grade 3) ($n = 3$), acute renal failure (grade 3) ($n = 1$), allergic reaction (grade 3) ($n = 1$), and interstitial pneumonitis (grade 3) ($n = 1$). Finally, a total of 219 patients were included; 212 patients (97%) developed PIPN which was characterized by numbness and tingling, while 7 had no PIPN symptoms. The maximum severity of PIPN reached in each of the 212 patients was as follows: grade 1, 159 patients (75%); grade 2, 45 patients (21%); and grade 3, 9 patients (4%). Two patients needed dose modifications due to PIPN above grade 2. No patients postponed or skipped the scheduled PTX due to PIPN.

Baseline characteristics of the population are listed in Table 1. The median age of patients was 53 years (range 22–70). Eighteen patients had diabetes mellitus without neuropathy complications at baseline. Disease-free survival and overall survival were evaluated with a median follow-up time of 57.1 months (range 5.3–95.5). A total of 25 patients received weekly PTX: 23 following AC and 2 without AC. The remaining 194 patients received tri-weekly PTX: 182 following AC and 12 without AC. The mean dose intensity was 58 mg/week (range 16–80). Treatment cessation was deemed necessary in 9 patients (4%); reasons for cessation were PIPN (8 patients, 3 with grade 1, 1 with grade 2, and 5 with grade 3) and myelosuppression (1 patient).

Table 1 Patient characteristics

Variables	triPTX ($N = 188$)	wPTX ($N = 24$)	All ($N = 212$)
Age			
Median (range)	53 (22–70)	52 (32–68)	53 (22–70)
<60 (%)	141 (75.0)	17 (70.8)	158 (74.5)
≥60 (%)	47 (25.0)	7 (29.2)	54 (25.5)
Sex (%)			
Female	187 (99.5)	24 (100.0)	211 (99.5)
Male	1 (0.5)	0 (0.0)	1 (0.5)
Lymph (%)			
<4	118 (62.8)	12 (50.0)	130 (61.3)
≥4	70 (37.2)	12 (50.0)	82 (38.7)
Tumor size (%)			
<5 cm	153 (81.4)	18 (75.0)	171 (80.7)
≥5 cm	35 (18.6)	6 (25.0)	41 (19.3)
Surgery (%)			
Mastectomy	114 (60.3)	16 (66.7)	130 (61.3)
Lumpectomy	73 (39.2)	8 (33.3)	81 (38.2)
Excisional biopsy	1 (0.5)	0 (0.0)	1 (0.5)
Systemic therapy (%)			
Chemo	56 (29.8)	8 (33.3)	64 (30.2)
Chemo + endocrine	132 (70.2)	16 (66.7)	148 (69.8)
Radiation (%)			
No	69 (36.7)	8 (33.3)	77 (36.3)
Yes	119 (63.3)	16 (66.7)	135 (63.7)
Hormone (%)			
Negative	48 (25.5)	5 (20.8)	53 (25.0)
Positive	140 (74.5)	19 (79.2)	160 (75.0)
HER2 (%)			
Negative	156 (83.0)	16 (66.7)	172 (81.1)
Positive	32 (17.0)	8 (33.3)	40 (18.9)
Diabetes mellitus (%)			
No	171 (91.0)	23 (95.8)	194 (91.5)
Yes	17 (9.0)	1 (4.2)	18 (8.5)

triPTX tri-weekly paclitaxel, *wPTX* weekly paclitaxel, *chemo* chemotherapy

PIPN development time

The median time taken for the total patient group to develop PIPN was 21 days (range 11–101) (Fig. 1). With weekly administration of PTX, the median time taken to develop PIPN was also 21 days (range 11–101); the median time with tri-weekly administration was 35 days (range 14–77).

Cumulative dose

The mean cumulative dose at the onset of grade 1 or higher PIPN was 175 mg/m^2 for patients treated with PTX every 3 weeks and 320 mg/m^2 for weekly PTX patients.

Diabetes mellitus

Of 18 diabetic patients, all had PIPN and 3 had maximum grade 3 PIPN. Median time to PIPN onset was 21 days (range 20–21), and median duration of PIPN was 287 days (range 70–503). In patients without diabetes, median time to PIPN was 21 days (range 20–21), and median duration of PIPN was 231 days (range 190–271).

Risk factors correlated with PIPN

Multivariate analysis using a logistic regression model after stepwise selection revealed no significant correlations between time to PIPN onset and maximum PIPN severity (Table 2), while there were significant correlations between duration of PIPN and age (>60 years old) ($P = 0.027$) and between duration of PIPN and maximum PIPN severity ($P = 0.015$) (Table 3). Moreover, we could not identify

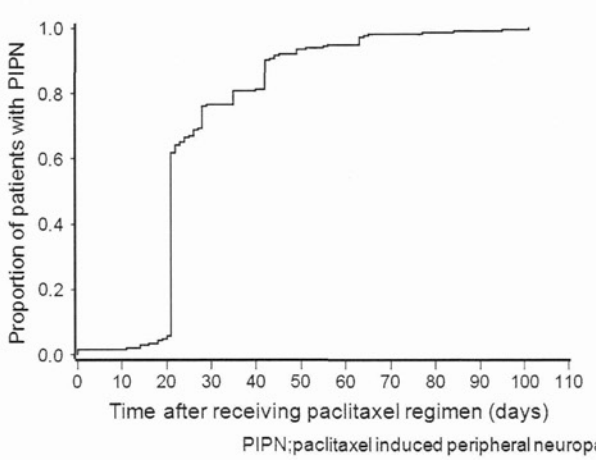

Fig. 1 Time taken for the total patient group to develop paclitaxel-induced peripheral neuropathy

Table 2 Multivariate analysis for factors associated with time to PIPN

Variables	HR	95% CI		*P* value
Regimen				
triPTX	1			
wPTX	0.66	0.43	1.03	0.070
Age				
<60				
≥60	0.99	0.72	1.37	0.960
Lymph				
<4				
≥4	1.20	0.82	1.77	0.341
Tumor size (cm)				
<5				
≥5	0.98	0.68	1.42	0.917
Radiation				
No				
Yes	0.78	0.51	1.20	0.259
Surgery				
Mastectomy				
Lumpectomy	1.08	0.75	1.56	0.666
Endocrine				
No				
Yes	0.87	0.65	1.18	0.366
Grade				
1				
2 or 3	1.35	0.97	1.87	0.073
Diabetes mellitus				
No				
Yes	1.34	0.81	2.21	0.260

PIPN paclitaxel-induced peripheral neurotoxicity, *triPTX* tri-weekly paclitaxel, *wPTX* weekly paclitaxel, *HR* hazard ratio, *CI* confidence interval

Table 3 Multivariate analysis for factors associated with duration of PIPN

Variables	HR	95% CI		*P* value
Regimen				
triPTX	1			
wPTX	0.48	0.19	1.21	0.119
Age				
<60				
≥60	0.55	0.32	0.94	0.027
Lymph				
<4				
≥4	0.86	0.46	1.59	0.621
Tumor size (cm)				
<5				
≥5	1.03	0.59	1.77	0.927
Radiation				
No				
Yes	1.05	0.52	2.12	0.900
Surgery				
Mastectomy				
Lumpectomy	0.67	0.36	1.26	0.213
Endocrine				
No				
Yes	1.10	0.70	1.73	0.668
Grade				
1				
2 or 3	0.53	0.32	0.88	0.015
Diabetes mellitus				
No				
Yes	0.66	0.28	1.56	0.345

PIPN paclitaxel-induced peripheral neurotoxicity, *triPTX* tri-weekly paclitaxel, *wPTX* weekly paclitaxel, *HR* hazard ratio, *CI* confidence interval

any correlation with grade 2/3 PIPN (Table 4). Based on the results of multivariate analyses, there were no significant associations between diabetes mellitus and time to PIPN onset ($P = 0.260$) or duration of PIPN ($P = 0.345$) or grade 2/3 PIPN ($P = 0.229$).

Duration of PIPN

The median duration of PIPN was 727 days for the total patient group (range 14–2621) (Fig. 2). With weekly administration, the median duration was not reached (range 14–1089); the median duration for patients with tri-weekly administration was 651 days (range 23–2621). One year after initiating PTX treatment, PIPN (all grades included) persisted in 64% of patients; 3 years after treatment initiation, this number had dropped to 41%.

Discussion

This is the first published report to our knowledge that investigates the time to onset and duration of PIPN among breast cancer patients and explores potential risk factors related to severe and/or persistent PIPN. The data from this study confirm that most patients (97%) developed PIPN with a severity of at least grade 1. Peripheral neuropathy persisted in 64% of patients at 1 year and 41% at 3 years after the first administration of PTX. Approximately half of the patients who received PTX and developed PN experienced recovery from PN within 9 months after cessation of PTX treatment. We found correlations between the maximum PIPN severity and both the time to onset of PIPN and the duration of PIPN. In addition, we observed that PN lasted significantly longer in patients >60 years of age.

Table 4 Multivariate analysis for factors associated with grade 2 or 3 PIPN

Variables	Odds ratio	95% CI		*P* value
Regimen				
triPTX	0.57	0.18	1.83	0.345
wPTX				
Age				
<60	1.65	0.81	3.36	0.171
≥60				
Lymph				
<4	0.98	0.40	2.41	0.968
≥4				
Tumor size (cm)				
<5	0.47	0.18	1.24	0.125
≥5				
Radiation				
No	0.98	0.35	2.77	0.975
Yes				
Surgery				
Mastectomy	0.73	0.29	1.82	0.499
Lumpectomy				
Endocrine				
No	0.72	0.36	1.45	0.360
Yes				
Diabetes mellitus				
No	2.05	0.69	6.09	0.197
Yes				
Dose intensity				
<58	1.00	0.50	2.01	1.000
≥58				
Cumulative dose				
<700	0.31	0.08	1.13	0.077
≥700	0.57	0.18	1.83	0.345

PIPN paclitaxel-induced peripheral neurotoxicity, *triPTX* tri-weekly paclitaxel, *wPTX* weekly paclitaxel, *CI* confidence interval

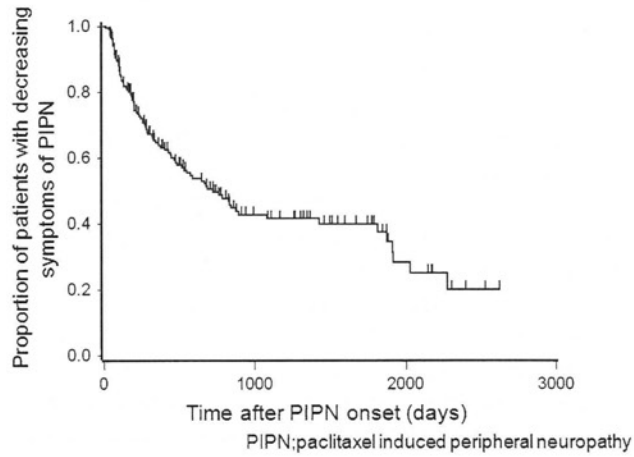

Fig. 2 Time to resolving PIPN from the time of developing paclitaxel-induced peripheral neuropathy

Previous studies have reported that the incidence of PIPN is related to several risk factors, including treatment schedule, doses per course, patient age, diabetes mellitus, and cumulative dose [6–11]. We found no association between the severity of PIPN and the PTX administration schedule including single dose, dose intensity, diabetes mellitus, or interval of administration. In our study, the mean cumulative dose at the onset of grade 1 or higher PN was 175 mg/m^2 for patients treated with PTX every 3 weeks and 320 mg/m^2 for weekly PTX patients. In contrast to an earlier study [14], our clinical outcomes indicated that tri-weekly administration of PTX was associated with more severe PIPN than weekly administration. However, this result may be attributed to frequent hospital visits and/or the relatively small number of patients treated by weekly PTX.

Previous reports suggest there are several risk factors for PIPN, including concurrent administration of cisplatin [19] and various genetic predispositions for neuropathy, such as *Wlds* (slow Wallerian degeneration gene) and *CYP3A* genotype [20, 21], but we did not examine any of those risk factors in this study.

Axonal microtubules are composed largely of β-tubulin. Neurotoxicity is caused by disruption of the microtubule structure, impairing axoplasmic transport and leading to dying-back neuropathy [22]. The most widely accepted mechanism of taxane neurotoxicity is a dying-back process that starts from distal nerve endings and progresses to affect Schwann cells, neuron bodies, or axons, resulting in transport changes that disturb cytoplasmic flow in the affected neurons [23]. Another possible cause of PIPN is that sensory nerves may be particularly vulnerable to the inhibition of tubulin assembly, as sensory nerves have long axons. However, motor neurons and C-neurons are not as sensitive to taxanes as are sensory nerves, despite the fact that these neurons are as long as sensory nerves. Some reports suggest that induction of Ca$\alpha 2\delta$-1 expression by PTX in the spinal root may be important, but further investigation is necessary to understand the mechanisms of PIPN [24].

There are no medications that prevent or relieve PIPN. Likewise, there are no laboratory tests that can predict the severity of PN. Management of PIPN is now based on early detection during chemotherapy to prevent its progression to grade 3 or 4. Clinical assessment, including a physical examination, is currently the most reliable method of assessing PIPN because we lack more reliable objective methods, and the symptoms of PIPN, such as numbness, sensory pain, fatigue, and weakness, are complicated [12, 25]. If grade 2 PN is diagnosed, it may be prudent to

withhold PTX until PN improves to at least grade 1; PTX administration can then be resumed at a reduced dose.

There were several limitations to our study. We used physician-based assessments, which relies on patients' report and examiners' interpretation and could have resulted in underestimation and under-reporting of the frequency and severity of PN [26]. In addition, physicians were more prone to quit following symptoms periodically once patients recovered from maximum PIPN. In fact, there were many censored cases in this study (Fig. 2). Therefore, features of PIPN such as location, presence of accompanying symptoms, and triggers for increase or decrease in severity were unclear. This study was retrospective, with censored data; the neurotoxicity corresponding to each grade of PIPN was unclear. In fact, time to onset of PIPN was faster for grades 2 and 3 than grade 1. In order to properly evaluate the correlation between severity and duration of PIPN, we will need further studies to determine whether or not the duration of PIPN is longer when the maximum severity increases from grade 1 to grade 2.

In conclusion, we analyzed the incidence and duration of PIPN and identified correlations between these and several risk factors. We found that the median time to onset of PIPN was 21 days, and the median duration of PIPN was 727 days. Patient age and PIPN severity were the independent risk factors significantly associated with longer PIPN duration. Urgent needs currently include identification of specific risk factors for PIPN, establishment of subjective methods for evaluating PIPN, and development of effective strategies for prevention and treatment of PIPN. To meet these ends, further investigation of the biological mechanisms leading to PIPN is warranted.

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Conflict of interest The authors have declared no conflicts of interest.

References

1. Henderson IC, Berry DA, Demetri GD et al (2003) Improved outcomes from adding sequential paclitaxel but not from escalating doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. J Clin Oncol 21:976–983
2. Mamounas EP, Bryant J, Lembersky B et al (2005) Paclitaxel after doxorubicin plus cyclophosphamide as adjuvant chemotherapy for node-positive breast cancer: results from NSABP B-28. J Clin Oncol 23:3686–3696
3. Buzdar AU, Singletary SE, Valero V et al (2002) Evaluation of paclitaxel in adjuvant chemotherapy for patients with operable breast cancer: preliminary data of a prospective randomized trial. Clin Cancer Res 8:1073–1079
4. De Laurentiis M, Cancello G, D'Agostino D et al (2008) Taxane-based combinations as adjuvant chemotherapy of early breast cancer: a meta-analysis of randomized trials. J Clin Oncol 26:44–53
5. Rowinsky EK, Donehower RC (1995) Paclitaxel (Taxol). N Engl J Med 332:1004–1014
6. Akerley W, Herndon JE, Egorin MJ et al (2003) Weekly, high-dose paclitaxel in advanced lung carcinoma: a phase II study with pharmacokinetics by the Cancer and Leukemia Group B. Cancer 97:2480–2486
7. Winer EP, Berry DA, Woolf S et al (2004) Failure of higher-dose paclitaxel to improve outcome in patients with metastatic breast cancer: Cancer and Leukemia Group B trial 9342. J Clin Oncol 22:2061–2068
8. Nabholtz JM, Gelmon K, Bontenbal M et al (1996) Multicenter, randomized comparative study of two doses of paclitaxel in patients with metastatic breast cancer. J Clin Oncol 14:1858–1867
9. Gogas H, Shapiro F, Aghajanian C et al (1996) The impact of diabetes mellitus on the toxicity of therapy for advanced ovarian cancer. Gynecol Oncol 61:22–26
10. Rowinsky EK, Eisenhauer EA, Chaudhry V et al (1993) Clinical toxicities encountered with paclitaxel (Taxol). Semin Oncol 20:1–15
11. Rowinsky EK, Chaudhry V, Cornblath DR et al (1993) Neurotoxicity of taxol. J Natl Cancer Inst Monogr 15:107–115
12. Lee JJ, Swain SM (2006) Peripheral neuropathy induced by microtubule-stabilizing agents. J Clin Oncol 24:1633–1642
13. Seidman AD, Berry D, Cirrincione C et al (2008) Randomized phase III trial of weekly compared with every-3-weeks paclitaxel for metastatic breast cancer, with trastuzumab for all HER-2 overexpressors and random assignment to trastuzumab or not in HER-2 nonoverexpressors: final results of Cancer and Leukemia Group B protocol 9840. J Clin Oncol 26:1642–1649
14. Sparano JA, Wang M, Martino S et al (2008) Weekly paclitaxel in the adjuvant treatment of breast cancer. N Engl J Med 358:1663–1671
15. Jones SE, Erban J, Overmoyer B et al (2005) Randomized phase III study of docetaxel compared with paclitaxel in metastatic breast cancer. J Clin Oncol 23:5542–5551
16. Goldhirsch A, Wood WC, Gelber RD et al (2007) Progress and promise: highlights of the international expert consensus on the primary therapy of early breast cancer 2007. Ann Oncol 18:1133–1144
17. Goldhirsch A, Ingle JN, Gelber RD et al, Panel members (2009) Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2009. Ann Oncol 20:1319–1329
18. Trotti A, Colevas AD, Setser A et al (2003) CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. Semin Radiat Oncol 13:176–181
19. Chaudhry V, Rowinsky EK, Sartorius SE et al (1994) Peripheral neuropathy from Taxol and cisplatin combination chemotherapy: clinical and electrophysiological studies. Ann Neurol 35:304–311
20. Wang MS, Davis AA, Culver DG et al (2002) WldS mice are resistant to paclitaxel (Taxol) neuropathy. Ann Neurol 52:442–447
21. Aplenc R, Glatfelter W, Han P et al (2003) CYP3A genotypes and treatment response in paediatric acute lymphoblastic leukaemia. Br J Haematol 122:240–244
22. Siau C, Bennett GJ (2006) Dysregulation of cellular calcium homeostasis in chemotherapy-evoked painful peripheral neuropathy. Anaesth Analg 102:1485–1490

23. Argyriou AA, Koltzenburg M, Polychronopoulos P et al (2008) Peripheral nerve damage associated with administration of taxanes in patients with cancer. Crit Rev Oncol Hematol 66:218–228
24. Gauchan P, Andoh T, Ikeda K et al (2009) Mechanical allodynia induced by paclitaxel, oxaliplatin and vincristine: Different effectiveness of gabapentin and different expression of voltage dependent calcium channel $\alpha 2\delta$-1 subunit. Biol Pharm Bull 32:732–734
25. Cavaletti G, Frigeni B, Lanzani F et al (2010) Chemotherapy-induced peripheral neurotoxicity assessment: a critical revision of the currently available tools. Eur J Cancer 46:479–494
26. Postma TJ, Heimans JJ, Muller MJ et al (1998) Pitfalls in grading severity of chemotherapy-induced peripheral neuropathy. Ann Oncol 9:739–744

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Original article

The differences in the histological types of breast cancer and the response to neoadjuvant chemotherapy: The relationship between the outcome and the clinicopathological characteristics

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ABSTRACT

Although effective regimens have been established for invasive ductal carcinoma-not otherwise specified (IDC), the efficacy and prognosis of other minor types of breast cancer are unknown because of their rareness. The clinicopathological features and prognosis of other minor types concerning the response to neoadjuvant chemotherapy (NAC) were evaluated in this study.

A total of 562 patients were classified according to the Japanese and the World Health Organization (WHO) classifications, and the number of IDC and other special types (SP) was 500 and 62. The SP patients had a significantly poorer clinicopathological response to NAC and less breast-conservative therapy than those with IDC. According to the WHO classification, mucinous carcinoma, metaplastic carcinomas and apocrine carcinoma also responded poorly, and patients with metaplastic carcinomas and invasive lobular carcinoma had a significantly poorer prognosis. Despite the poor response to chemotherapy, patients with mucinous carcinoma and apocrine carcinoma had a good prognosis.

The response to NAC and the prognosis vary for each histological type. For some types, the prognosis was not related to the clinicopathological response to NAC.

Background: In the treatment of breast cancer, neoadjuvant chemotherapy (NAC) has become the standard treatment modality for downstaging purposes. Although effective regimens have been established for the treatment of invasive ductal carcinoma-not otherwise specified (IDC), the data about the efficacy and prognosis for patients with other minor types of breast cancer are insufficient because of the rareness of these tumors. Defining the relationship between each histological type and the clinicopathological response to NAC is essential to optimizing individualized treatment.

Methods: We retrospectively evaluated the clinicopathological features and classification of the histological types based on the Japanese and the World Health Organization (WHO) classifications before and after NAC in 562 patients with primary breast cancer who underwent curative treatment after NAC between 1998 and 2008. The prognosis was estimated for each histological type.

Results: Of the 562 patients, the number of cases of IDC and other special types (SP) was 500 and 62. In the SP group, the clinicopathological response to NAC was significantly poorer, and the patients underwent breast-conservative therapy less frequently than did the IDC patients. According to the WHO classification, mucinous carcinoma, metaplastic carcinomas and apocrine carcinoma responded poorly to NAC. The disease-free survival and overall survival were significantly worse for patients with metaplastic carcinomas ($p < 0.001$ and $p < 0.001$) and with invasive lobular carcinoma ($p = 0.03$ and $p < 0.001$) than other cancers. Despite their poor response to treatment, patients with mucinous carcinoma and apocrine carcinoma had a good prognosis.

Conclusions: The response to standardized NAC and prognosis varies for each histological type. For some types, the prognosis was not associated with the clinicopathological response to NAC. Innovative regimens should therefore be investigated for each histological type to achieve the best response.

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Introduction

In the treatment of breast cancer, neoadjuvant chemotherapy (NAC) has become the standard treatment modality for down-staging purposes. With the introduction of NAC, many patients have been able to be treated with breast-conserving therapy (BCT) as a result of the tumor reduction prior to surgery. Especially for patients with invasive ductal carcinoma-not otherwise specified (IDC), NAC had been confirmed to be efficient and beneficial, and is now widely applied for treatment. At present, invasive breast carcinoma is treated with a standardized regimen of NAC, regardless of the pathological type. However, because of their rareness, the efficiency and outcomes of NAC for the other minor types of breast carcinoma have not been fully elucidated.

In this study, we made a comparison between the patients with IDC and other types of breast cancer about clinicopathological features with regard to NAC. The histological types were classified using the Japanese classification[1,2] and the World Health Organization (WHO) classification.[3] We have correlated these histological types with the overall survival (OS) and disease-free survival (DFS) of the patients, and assessed the association between the tumor response to standardized NAC and the outcome for each histological type.

Material and methods

Patients

This study was a retrospective analysis of 562 breast cancer patients who underwent NAC during the period from 1998 to 2008 at the National Cancer Center Hospital, Tokyo, Japan. NAC was indicated for clinical stage II tumors that were larger than 3 cm in diameter, and for all stage III tumors. Axillary lymph node metastasis was diagnosed by cytology or imaging studies. Prior to NAC, all the patients underwent a core needle biopsy (CNB) for histological examination and were staged according the International Union Against Cancer (UICC) TNM classification.

Neoadjuvant chemotherapy regimens

NAC regimens were introduced based on current reviews at the time. Anthracycline-based chemotherapy included four cycles of CEF (cyclophosphamide 500 mg/m^2, epirubicin 100 mg/m^2, and fluorouracil 500 mg/m^2) every 3 weeks, or four cycles of AC (doxorubicin 60 mg/m^2, and cyclophosphamide 600 mg/m^2). Taxane chemotherapy included 12 cycles of weekly paclitaxel (wPTX, 80 mg/m^2).[4,5] Concurrent anthracycline and taxane chemotherapy included four cycles every 3 weeks of doxorubicin and docetaxel (AT, 50 and 60 mg/m^2).[6] Sequential anthracycline and taxane chemotherapy included AT (two cycles) followed by wPTX, AC followed by wPTX, and CEF followed by wPTX. Trastuzumab (first cycle; 4 mg/kg: after second cycle; 2 mg/kg) combined with anthracycline and taxane chemotherapy was administered to the patients with overexpression of the human epidermal growth factor receptor 2 (HER2).[7]

Histological diagnosis and evaluation

Prior to NAC, CNB specimens were examined for the histological sub-type and histological grade (HG) by hematoxylin and eosin (HE) staining. After NAC, the surgical specimen was examined for the histological sub-type, HG, and presence or absence of lymphatic or vascular space invasion. The histological sub-types were defined based on the General Rules for Clinical and Pathological Recording of Breast Cancer that were proposed by The Japanese Breast Cancer Society (JBCS classification)[1,2] and the WHO classification.[3] As the feature of the Japanese histological classification, all breast carcinomas are first classified according to the existence of invasion while, in addition, invasive carcinoma is classified as invasive ductal carcinoma, or other types called 'special types (SP)', and the SP category includes invasive lobular carcinoma (ILC) and other minor histological types.

The HG was assessed using the Scaff-Bloom-Richardson classification.[8] Immunohistochemistry was used to examine the tissue samples for the expression of the estrogen receptor (ER), progesterone receptor (PgR), and HER2. The cutoff values for the ER and PgR were 10% positive cells. HER2 status was defined based on immunohistochemical staining (IHC). The specimens that were HER2 2 + by IHC were then subjected to fluorescence *in situ* hybridization (FISH). HER2 positive samples were defined as those that were HER2 3+ in IHC or HER2 2+ in IHC and had an amplification ratio in FISH of >2.0. The degree of lymphatic invasion (ly) was classified by HE staining as follows: absent, no lymphatic invasion; ly1+, minimal lymphatic invasion; ly2+, moderate lymphatic invasion; and ly3+, marked lymphatic invasion. These diagnoses and evaluations were performed separately by two qualified pathologists, and the final diagnosis and evaluations were decided as a result of conferences between the pathologists.

Evaluation of the response to NAC

Prior to and after NAC, all of the patients and tumors were evaluated by physical examinations and radiographic imaging. The tumor diameter was evaluated using calipers and by ultrasonography. The clinical response was assessed using the Response Evaluation Criteria in Solid Tumor (RECIST) guidelines.[9] The tumor was judged to be 'progressive disease (PD)' when the tumor size increased by 20% or more. At that time, chemotherapy was discontinued and surgery was performed. The pathological response was evaluated from surgical specimens. The histopathological response was assessed using the General Rules for Clinical and Pathological Recording of Breast Cancer.[10] Response grade 0 was no response, and was defined by almost no change in the cancer cells after treatment. Grade 3 was a complete response, and was defined as necrosis or the disappearance of all tumor cells. The definition of a pathological complete response (pCR) was 'necrosis and the disappearance of all invasive cells' of the primary tumor. Cases with only intraductal carcinoma remaining were included in the pCR category.

Table 1
The Japanese histological classification of breast tumors (extraction) and the number of patients with each histological type ($n = 562$).

Histological type	No. of patients	%
B. Malignant (Carcinoma)		
a. Invasive carcinoma	500	89.0
a1. Papillotubular carcinoma	126	22.4
a2. Solid-tubular carcinoma	202	35.9
a3. Scirrhous carcinoma	172	30.6
b. Special types	62	11.0
b1. Mucinous carcinoma	12	2.1
b2. Medullary carcinoma	0	0
b3. Invasive lobular carcinoma	29	5.2
b4. Adenoid cystic carcinoma	0	0
b5. Squamous cell carcinoma	5	0.9
b6. Spindle cell carcinoma	4	0.7
b7. Apocrine carcinoma	5	0.9
b8. Carcinoma with cartilaginous and/or osseous metaplasia	1	0.2
b9. Tubular carcinoma	0	0
b10. Secretory carcinoma	1	0.2
b11. Invasive micropapillary carcinoma	1	0.2
b12. Matrix-producing carcinoma	4	0.7
b13. Others	0	0

Table 2
The administered NAC regimens ($n = 562$).

	No. of patients	%
AT	150	26.7
AT followed by wPTX	25	4.4
AT followed by wPTX/Trastuzumab	2	0.4
AC followed by wPTX	142	25.3
AC followed by wPTX/Trastuzumab	17	3.0
CEF followed by wPTX	181	32.2
CEF followed by wPTX/Trastuzumab	26	4.6
wPTX	12	2.1
wPTX/Trastuzumab	7	1.2

AT, doxorubicin and docetaxel; wPTX, weekly paclitaxel; AC, doxorubicin and cyclophosphamide; CEF, cyclophosphamide, epirubicin and fluorouracil.

Surgery and post-operative treatment

The breast surgery was either a lumpectomy or a total mastectomy. When the patient who underwent a lumpectomy was detected to have cancer in the pathological margin, additional excision was performed until the specimen became pathologically margin free. All of the patients underwent axillary lymph node dissection (level II). Adjuvant therapy was given in some cases based on the most current recommendations from the St. Gallen's Consensus Meeting at the time.[11–15] Tamoxifen (20 mg/day) or anastrozole (1 mg/day) was administered for five years when CNB specimens or surgical postchemotherapy specimens were positive for the ER or PgR. Radiotherapy was performed for the patients who underwent BCT for the residual breast or the patients with tumors >5 cm and/or with massive metastatic lymph nodes (≥4 nodes) for the chest wall, axilla, and supraclavicular area.

Follow-up and statistical analysis

The number of follow-up months was recorded from the first day of NAC to the most recent medical visit on record.

OS and DFS were calculated using the Kaplan–Meier methods and compared using the log-rank test. For comparisons of categorical variables, the chi-square test was used. Odds ratios (OR) and associated 95% confidence intervals (95% CI) were calculated as estimates of the relative risk. Values of $p < 0.05$ were considered to be statistically significant. All data were analyzed using the SPSS software program (SPSS Inc., Chicago, IL).

Results

Patient characteristics and clinical features

Table 1 presents the Japanese histological classification and the number of each histological type. The total number of IDC and SP

Table 3
The results of the analysis of the patient and tumor characteristics by histological groups (JBCS).

	Univariate			Multivariate	
	IDC ($n = 500$)	SP ($n = 62$)	p value	OR (95% CI)	p value
Age, mean ± SD	50.7 ± 10.4	50.6 ± 11.7	0.932		
Age (years)			0.335		
<41	74 (14.8)	13 (21.0)			
41–50	147 (29.4)	15 (24.2)			
51–60	178 (0.356)	18 (29.0)			
≥61	101 (0.202)	16 (25.8)			
Tumor size (cm), mean ± SD					
Prior NAC	5.7 ± 1.7	5.5 ± 2.5	0.075		
After NAC	2.1 ± 1.9	3.5 ± 2.7	<0.001	1.318 (1.063–1.632)	0.012
Stage			0.841		
II	320 (64.0)	39 (62.9)			
III	180 (36.0)	23 (37.1)			
Hormone receptors					
ER positive (%)	223 (44.6)	27(43.5)	0.892		
PgR positive (%)	198 (39.6)	21 (33.9)	0.408		
HER2 positive (%)	105 (21.0)	4 (6.5)	0.006	0.275 (0.080–0.948)	0.041
Histological grade			<0.001	0.674 (0.403–1.125)	0.131
G1 (%)	32 (6.4)	14 (22.6)			
G2 (%)	216 (43.2)	27 (43.5)			
G3 (%)	252 (50.4)	21 (33.9)			
Clinical response					
Responded (CR + PR) (%)	425 (85.0)	42 (67.7)	0.002	0.841 (0.341–2.076)	0.707
CR	165 (33.0)	6 (9.6)	<0.001	0.938 (0.633–1.390)	0.750
PD	13 (2.6)	7 (11.3)	0.003	5.279 (1.715–16.249)	0.004
BCT cases (%)	208 (53.0)	16 (25.8)	0.019	0.386 (0.082–1.247)	0.240
Pathological response					
pCR	113 (22.6)	5 (8.1)	0.080		
Pathological response grade					
G0 (%)	15 (3.0)	6 (9.7)	0.021	2.911 (0.777–10.909)	0.113
G3 (%)	65 (13.0)	5 (8.1)	0.314		
G0/1 (%)	312 (62.4)	42 (67.7)	0.086		
G2/3 (%)	188 (37.6)	20 (32.3)			
Cases of LN metastasis (%)	265 (53.0)	38 (61.3)	0.227		
No. of LN metastasis, mean ± SD	2.8 ± 5.1	3.7 ± 6.6	0.215		
Lymphatic invasion					
present	133 (26.6)	12 (19.4)	0.042	0.385 (0.174–0.851)	0.018
ly(1+)	85 (17.0)	10 (16.1)	0.302		
ly(2+/3+)	48 (9.6)	2 (3.2)	0.018	0.324 (0.073–1.448)	0.140
Vascular invasion, present	17 (3.4)	2 (3.2)	1.000		

IDC, invasive ductal carcinoma-not otherwise specified; SP, special types; OR, odds ratio; CI, confidence interval; SD, standard deviation; NAC, neoadjuvant chemotherapy; ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2; G, grade; CR, complete response; PR, partial response; PD, progressive disease; BCT, breast-conserving therapy; pCR, pathological complete response; LN, lymph node.

Table 4
The results of the univariate analysis of the patient and tumor characteristics for each histological type (WHO).

	IDC ($n = 500$)	ILC($n = 29$)		Metaplastic ($n = 14$)		Mucinous ($n = 12$)		Apocrine ($n = 5$)	
			p value		p value		p value		p value
Tumor size (cm), mean + SD									
Prior NAC	5.0 + 1.7	4.7 + 1.2	0.169	5.9 + 2.9	0.254	7.4 + 3.4	0.036	5.3 + 1.4	0.709
After NAC	2.1 + 1.9	2.4 + 1.7	0.400	5.9 + 3.9	0.003	4.1 + 1.7	0.001	3.0 + 0.1	<0.001
Hormone receptors									
ER positive (%)	223 (44.6)	14 (50.0)	0.698	1 (7.1)	0.005	9 (75.0)	0.039	0 (0)	0.061
PgR positive (%)	198 (39.6)	13 (46.4)	0.556	1 (7.1)	0.011	6 (50.0)	0.348	1 (25.0)	0.163
HER2 positive (%)	105 (21.0)	2 (7.1)	0.091	0 (0)	0.085	1 (8.3)	0.252	1 (20.0)	0.999
Histological grade			0.069		0.016		<0.001		0.372
G1	32 (6.4)	4 (14.3)		0 (0)		8 (66.7)		1 (20.0)	
G2	216 (43.2)	17 (60.7)		1 (7.1)		3 (25.0)		4 (80.0)	
G3	252 (50.4)	7 (25.0)		13 (92.9)		1 (8.3)		0 (0)	
Clinical response									
Responded (CR + PR)	425 (85.0)	21 (75.0)	0.211	5 (35.7)	0.003	9 (75.0)	0.271	5 (100)	0.446
CR	165 (33.0)	5 (17.9)	0.067	0 (0)	0.007	0 (0)	0.009	0 (0)	0.137
PD	13 (2.6)	0 (0)	0.428	7 (50.0)	<0.001	0 (0)	0.603	0 (0)	0.737
Pathological response									
pCR	113 (22.6)	2 (7.1)	0.032	0 (0)	0.047	0 (0)	0.045	0 (0)	0.272
Pathological response grade			0.357		0.094		0.116		0.372
G0/1	312 (62.4)	19 (67.9)		12 (85.7)		10 (83.3)		2 (40.0)	
G2/3	188 (37.6)	9 (32.1)		2 (14.3)		2 (16.7)		3 (60.0)	
Cases of LN metastasis	265 (53.0)	19 (67.9)	0.089	7 (50.0)	0.968	9 (75.0)	0.111	1 (20.0)	0.194
No. of LN metastasis, mean + SD	2.8 + 5.1	4.4 + 6.8	0.223	5.2 + 9.5	0.341	1.7 + 2.0	0.453	0.2 + 0.4	0.260
Lymphatic invasion, present	133 (26.6)	4 (14.2)	0.307	3 (21.4)	0.385	4 (33.3)	0.771	0 (0)	0.161
Vascular invasion, present	17 (3.4)		0.357	0 (0)	1.000	0 (0)	1.000	0 (0)	1.000

IDC, invasive ductal carcinoma-not otherwise specified; ILC, invasive lobular carcinoma; SD, standard deviation; NAC, neoadjuvant chemotherapy; ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2; G, grade; CR, complete response; PR, partial response; PD, progressive disease; pCR, pathological complete response; LN, lymph node.

cases was 500 and 62. Table 2 shows the NAC regimens that were administered. Prior to NAC, the average age and tumor size were not significantly different for the different groups. The HG was significantly higher in the IDC group ($p < 0.001$). The immunohistochemical findings and ER and PgR status were not significantly different in the two groups, however, the HER2 status was more frequently positive in the IDC group ($p = 0.006$). After NAC, the SP group was significantly less likely to achieve a clinical response ($p = 0.002$) and had tumors that were larger in size ($p < 0.001$). There were 20 patients who discontinued NAC because of PD. This was 11.3% of the cases in the SP group, which was significantly higher ($p = 0.003$) than that in the IDC group. BCT was performed significantly more often for IDC patients than SP patients (53.0% vs. 25.8%, $p = 0.019$). Axillary lymph node metastasis was present in 53.0% of patients in the IDC group and 61.3% of those in the SP group, which was not significantly different. The average number of metastatic lymph nodes was not significantly different between the groups. With regard to the pathological response, the pCR rate was 22.6% in the IDC group and 8.1% in the SP group, which was not significantly different. The rate of pathological response grade 3 was also not significantly different between the groups. However, 9.7% of SP patients had no pathological response, and this was a significantly higher rate than that in IDC patients ($p = 0.021$). The IDC group had larger tumors ($p = 0.042$), and more severe ($p = 0.018$) lymphatic invasion. The frequency of vascular invasion was not significantly different between the groups. According to a multivariate analysis, the significantly different characteristics in the SP group were a larger tumor size after NAC, more frequent HER2-negative status, more PD and a lower severity of lymphatic invasion (Table 3).

Histological classification and clinicopathological response to NAC

According to the WHO classification, squamous cell carcinoma, spindle cell carcinoma, carcinoma with cartilaginous and/or osseous metaplasia, and matrix-producing carcinoma were included in the category of metaplastic carcinomas (MPC). The total number of MPC was 14 cases. The tumor size of mucinous carcinomas, MPC and apocrine carcinomas was only minimally reduced, and this was significantly different from IDC ($p = 0.001$, $p = 0.003$ and $p < 0.001$). The clinical response of MPC was significantly poorer than that of IDC ($p = 0.003$) and a half of MPC cases

Table 5
The results of the multivariate analysis of the patient and tumor characteristics of patients with metaplastic carcinomas and mucinous carcinoma (WHO).

	Metaplastic		Mucinous	
	OR (95% CI)	p value	OR (95% CI)	p value
Tumor size, Prior NAC			1.416 (0.983–2.041)	0.062
Tumor size, After NAC	1.443 (1.065–1.956)	0.018	1.226 (0.765–1.964)	0.398
ER positive	0.122 (0.012–1.265)	0.079	1.746 (0.350–8.703)	0.496
PgR positive	0.389 (0.042–3.603)	0.406		
Histological grade	5.935 (0.709–49.680)	0.100	0.077 (0.021–0.280)	<0.001
Clinical response, (CR + PR)	0.545 (0.125–2.367)	0.418		
Clinical response, CR	0.117 (0.001–35.290)	0.830	0.071 (0.001–20.076)	0.861
Clinical response, PD	36.409 (3.408–289.011)	0.003		
Pathological response, pCR	0.028 (0.001–27.724)	0.835	0.003 (0.001–17.390)	0.898

OR, odds ratio; CI, confidence interval; NAC, neoadjuvant chemotherapy; ER, estrogen receptor; PgR, progesterone receptor; CR, complete response; PR, partial response; PD, progressive disease; pCR, pathological complete response.

developed PD, which was significantly higher than the rate of IDC ($p < 0.001$). The HG was lower in mucinous carcinomas ($p < 0.001$) and higher in cases of MPC ($p = 0.016$) than in IDC (Table 4). A multivariate analysis indicated that mucinous carcinoma had a lower HG and that MPC had a larger tumor size after NAC and more frequently developed PD than did patients with IDC (Table 5).

Prognosis after treatment and histological features

The patient survival was evaluated using a median follow-up period of 49 months (range, 1–136 months). The 10 year DFS rate was 28% in the SP group and 62% in the IDC group ($p < 0.001$). The OS was significantly worse in the SP group than the IDC group ($p < 0.001$). The incidence of recurrence or death was also significantly higher in the SP group (OR. 2.359; 95% CI, 1.443–3.856; $p < 0.001$ and OR, 4.825; 95% CI, 2.473–9.412; $p < 0.001$, respectively). The independent risk of recurrence or death was analyzed using a Cox multivariate analysis (Table 6). The independent risk factors for recurrence were a younger age, a high HG and the presence of lymphatic invasion. The pathological response grade was a significant factor associated with OS. However, PD was not a significant factor for predicting the DFS or OS.

According to the WHO classification, the DFS and OS of MPC and ILC were significantly worse than those of IDC. However, there were no cases of recurrence or death in the patients with apocrine carcinoma (Fig. 1). The incidence of recurrence or death was significantly higher in the MPC group (OR, 3.076; 95% CI, 1.057–8.951; $p = 0.031$ and OR, 7.053; 95% CI, 2.347–21.197; $p < 0.001$, respectively). The other three types were not significantly different with regard to the incidence of recurrence or death. Because there was only a small number of cases of each histological type, no significant independent risk factor for recurrence or death were identified in the multivariate analysis of each histological type.

Discussion

For breast cancer patients, NAC has been standardized for the purpose of reducing the tumor or for downstaging the tumor. For IDC, standardized NAC regimens have been established, and the effects of treatment have been widely shown.[16,17] However, because of their rareness, the therapeutic effect and outcome after NAC for other types (excluding IDC) were unclear, and standardized regimens for each histological type have not been established. In Japan, standardized NAC was started in 1998, and has been administered for all types of invasive breast carcinoma. We have demonstrated that there are differences in the clinicopathological effects and outcomes after NAC for different types of invasive breast carcinoma, and that these differences are especially pronounced between IDC and other minor types based on the Japanese and the WHO classifications.

Although the SP group had a significantly poorer outcome with regard to tumor reduction and the pathological response, there were actually two sub-types of tumors; those that were effectively reduced by NAC (mucinous carcinoma, ILC and apocrine carcinoma) and those that increased in size despite treatment (squamous carcinoma and spindle cell carcinoma). Under the WHO classification, these increased types were included among the MPC group.

Overall, the SP group had a significantly poorer prognosis than the IDC group. However, according to the WHO classification, the SP group could be sub-classified into better and worse prognostic types, irrespective of the poor response to NAC. ILC and MPC had significantly poorer outcomes than IDC, but mucinous carcinoma and apocrine carcinoma did not have significant differences in their DFS and OS compared to IDC patients. These results suggest that the SP group in the JBCS classification includes different biological and clinical types.

The behavior and a better prognosis of mucinous carcinoma and apocrine carcinoma were reported.[18–21] Because of their better prognosis regardless of the little effect of NAC, the role of NAC for these carcinomas was limited and NAC might not be needed.

MPC was characterized that the biological and clinical malignancies,[22,23] and the subgroups of MPC included carcinoma with cartilaginous and/or osseous metaplasia and matrix-producing carcinoma were previously reported by Wargotz et al.[24–28] Because of its sarcomatous lesion, MPC has only a minimal response to NAC using the conventional regimens[29] and the effeteness of anti-sarcoma regimens including ifosfamide and etoposide was reported.[30] In our study, the clinicopathological characteristics and response to NAC were similar to other reports,[31–34] but the prognosis was poorer and different. From 1990

Table 6
The hazard ratio of the disease free interval and overall survival in patient with special types based on the multivariate Cox regression analysis.

	DFS			OS		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Age	0.898	0.832–0.969	0.005	0.979	0.885–1.082	0.673
Tumor size						
Prior NAC	0.874	0.537–1.424	0.589	1.273	0.884–2.415	0.084
After NAC	1.166	0.696–1.956	0.559	1.604	0.948–2.713	0.078
Stage	0.815	0.154–4.305	0.810	0.914	0.241–5.214	0.897
Hormone receptors						
ER positive	1.416	0.197–10.187	0.730	0.383	0.040–3.687	0.406
PgR positive	3.540	0.449–27.927	0.230	0.547	0.018–17.071	0.731
HER2 positive	0.007	0.001–3.142	0.974	0.071	0.001–4.682	0.991
Histological grade	6.022	1.458–24.864	0.013	3.195	0.312–31.992	0.330
Clinical response						
Responded (CR + PR)	0.480	0.029–7.985	0.609	0.555	0.072–40,281	0.572
CR	0.004	0.001–6.486	0.991	0.013	0.001–9.246	0.995
PD	4.628	0.353–60.629	0.243	4.560	0.221–92.262	0.326
Pathological response, pCR	0.871	0.001–17.512	1.000	0.653	0.032–12.486	0.998
Pathological response grade	0.754	0.314–1.811	0.528	0.339	0.117–0.983	0.046
Cases of LN metastasis	1.084	0.091–12.867	0.949	1.898	0.032–23.623	0.868
No. of LN metastasis	1.111	0.949–1.301	0.188	5.856	0.031–52.465	0.889
Lymphatic invasion, present	6.384	1.329–30.666	0.021	2.243	0.225–22.394	0.491
Vascular invasion, present	12.136	0.001–144.730	0.964	4.467	0.001–35.241	0.994

DFS, disease-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; NAC, neoadjuvant chemotherapy; ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2; CR, complete response; PR, partial response; PD, progressive disease; pCR, pathological complete response; LN, lymph node.

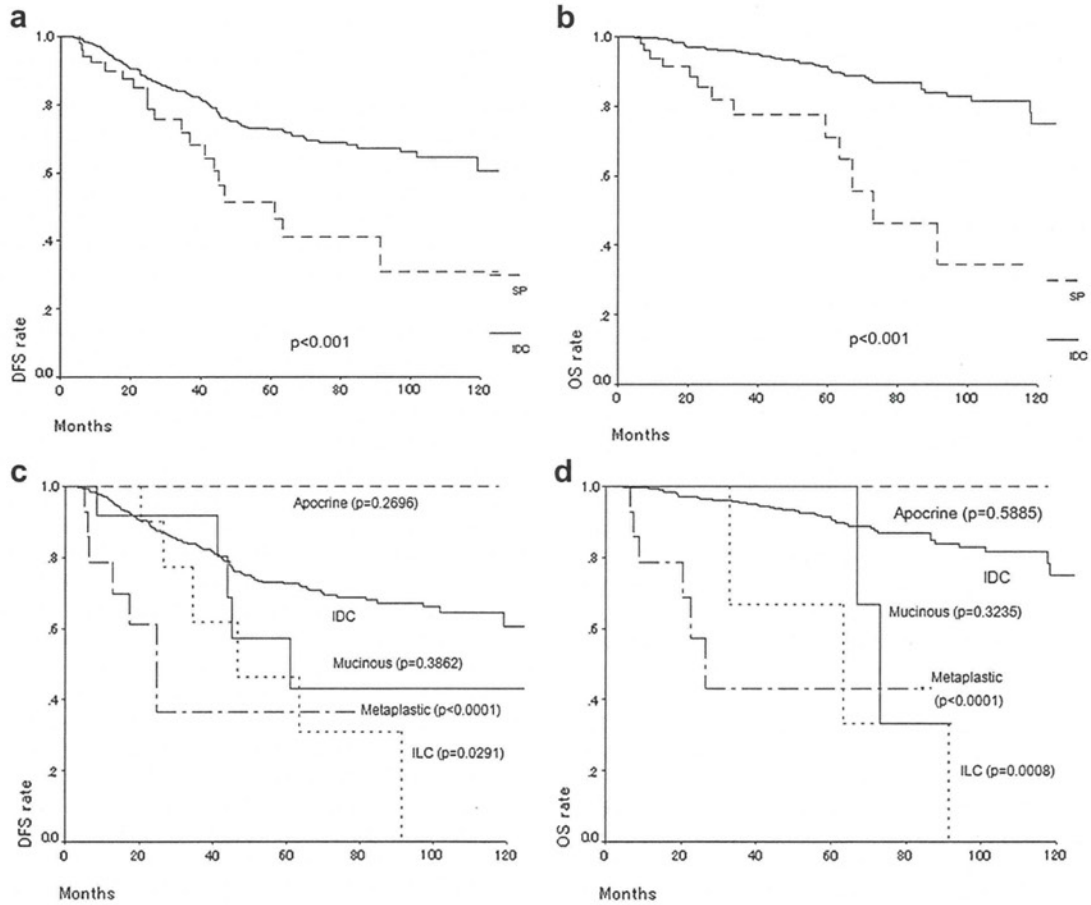

Fig. 1. The disease-free survival (DFS) curves and overall survival (OS) curves. (a) The DFS of IDC and SP patients based on the JBCS classification, (b) The OS of the IDC and SP patients, (c) The DFS for each histological type based on the WHO classification, (d) The OS for each histological type.

to 2009 at our institute, the 10-year survival rate of IDC and ILC patients were 81.8% and 76.5%, which was not significantly different. The reason for the relatively poor prognosis in our study is unclear, but it is possible that the chemosensitivity of ILC may differ in different races as a result of genetic differences.

Currently, breast cancer has been shown to be classifiable into molecular sub-types by gene profiling, and these sub-types related to different prognoses.[35,36] The use of adjuvant or neoadjuvant therapies has been shifting from an emphasis on the histological type to being based on the specific molecular sub-types. With regard to the molecular sub-types, positivity for the ER and/or PgR was not associated with any significant difference between the IDC and SP groups, but there were significantly more HER2-negative cases in the SP group. Several authors have reported that HER2-positive tumors were predicted to have an improved response to chemotherapy and to achieve a much higher pCR rate.[37,38] The HER2-negative status may be one reason why the SP group had a poorer overall response to NAC.

The relationship between chemosensitivity and the molecular sub-types has already clarified that ER-negative tumors have a good response to chemotherapy.[38,39] The molecular sub-types, prognosis and epidemiology of each rare histological type were reviewed by Yerushalmi et al.[40] From the analysis of the histological type in our study, the ER status was found to be positive in cases of mucinous carcinoma and negative in cases of MPC. ER positive status is considered to be the reason for the poor response of mucinous carcinoma. However, MPC had poor response to NAC regardless of the ER status, so the reason for the poorer prognosis is still unclear. MPC is considered to be a basal-like tumor because it is 'triple-negative', and this type has poor chemosensitivity and a poor prognosis.[41] In fact, all of the PD cases in our SP group were MPC. Because of their poor response, NAC is generally omitted for these patients, and surgical resection is performed as the primary therapy for mucinous carcinoma and MPC.

Besides molecular sub-types, other classifications, such as that using the 21-gene expression profile assay and 70-gene assay, have been used for predicting the response to neoadjuvant and adjuvant therapy.[42,43] Although a review concerning the relationship between neoadjuvant endocrine therapy and the 21-gene expression profile assay was reported from Japan,[44] this was a pilot study, and the scoring tools are not yet widespread because of the high price of employing this method. New therapeutic regimens based on the further analysis of the relationship between the immunohistological features or gene expression profiles and therapeutic sensitivity are thus needed.

Some of the limitations associated with this study are the fact that it was a retrospective analysis, and the study population was small due to the rareness of patients with each histological type in the SP group. Trastuzumab therapy was performed in only 52 cases, although there were 109 cases with HER2-positive tumors. The reason for this difference is the date of approval of trastuzumab in Japan. Chemotherapy regimens have been changed during the period of the study, and a uniform evaluation of the effects of therapy cannot be performed. Additionally, treatment for breast cancer has been changed dramatically in the past few years.[45] Because the basis of treatment has been changed from histopathological characteristics of tumor or the presence or absence of lymph node metastasis to intrinsic sub-type of tumor, the role of

chemotherapy has been getting smaller. Therefore the treatment criterion in this review may be different.

In summary, the other minor types of invasive breast carcinoma were different from IDC with regard to the effects of NAC and the prognosis. To determine whether NAC should be administered for the various sub-types of breast cancer, an accurate histological diagnosis and an appreciation of the individual sub-type's sensitivity and responsiveness to NAC are essential. Favorable chemotherapy regimens should be developed for each sub-type. For the types with poor response to NAC, innovative regimens based on their unique clinicopathological features should be investigated.

Conflict of interest

None declared.

References

1. The Japanese Breast Cancer Society. *General rules for clinical and pathological recording of breast cancer* (in Japanese). 16th ed. Tokyo: Kanehara; 2008. 18–25.
2. The Japanese Breast Cancer Society. Histological classification. *Breast Cancer* 2005;**12**(Suppl.):S12–4.
3. The World Health Organization. The World Health Organization histological typing of breast tumors second edition. *Am J Clin Pathol* 1983;**78**:806–16.
4. Buzdar AU, Singletary SE, Theriault RL, Booser DJ, Valero V, Ibrahim N, et al. Prospective evaluation of paclitaxel versus combination chemotherapy with fluorouracil, doxorubicin, and cyclophosphamide as neoadjuvant therapy in patients with operative breast cancer. *J Clin Oncol* 1999;**17**:3412–7.
5. Green MC, Buzdar AU, Smith T, Ibrahim NK, Valero V, Rosales MF, et al. Weekly paclitaxel improves pathologic complete remisson in operative breast cancer when compared with paclitaxel once every 3 weeks. *J Clin Oncol* 2005;**23**:5983–92.
6. Goldstein L, O'Neill A, Sparano J, Perez E, Shulman L, Martino S, et al. E2197: Phase III AT (doxorubicin/docetaxel) vs. AC (doxorubicin/cyclophosphamide) in the adjuvant treatment of node positive and high risk node negative breast cancer. *J Clin Oncol* 2005;**23**(June 1 Suppl.):16S. 512.
7. Burstein HJ, Harris LN, Gelman R, Lester SC, Nunes RA, Kaelin CM, et al. Preoperative therapy with trastuzumab and paclitaxel followed by sequential adjuvant doxorubicin/cyclophosphamide for HER2 overexpressing stage II or III breast cancer: a pilot study. *J Clin Oncol* 2003;**21**:46–53.
8. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histopathological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* 1991;**19**:403–10.
9. Therasse P, Arbuck SG, Eisenhauer EA, Wandews J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;**92**:205–16.
10. Kurosumi M, Akashi-Tanaka S, Akiyama F, Komoike Y, Mukai H, Nakamura S, et al. Histopathological criteria for assessment of therapeutic response in breast cancer (2007 version). *Breast Cancer* 2008;**15**:5–7.
11. Goldhirsch A, Glick JH, Gelber RD, Senn HJ. Meeting highlights: international consensus panel on the treatment of primary breast cancer. *J Natl Cancer Inst* 1998;**90**:1601–8.
12. Goldhirsch A, Glick JH, Gelber RD, Coates AS, Senn HJ. Meeting highlights: international consensus panel on the treatment of primary breast cancer. *J Clin Oncol* 2001;**19**:3817–27.
13. Goldhirsch A, Wood WC, Gelber RD, Coates AS, Thürlimann B, Senn HJ. Meeting highlights: updated international expert consensus on the primary therapy of early breast cancer. *J Clin Oncol* 2003;**21**:3357–65.
14. Goldhirsch A, Glick JH, Gelber RD, Coates AS, Thürlimann B, Senn HJ. Meeting highlights: international expert consensus on the primary therapy of early breast cancer 2005. *Ann Oncol* 2005;**16**:1569–83.
15. Goldhirsch A, Wood WC, Gelber RD, Coates AS, Thürlimann B, Senn HJ. Progress and promise: highlights of international expert consensus on the primary therapy of early breast cancer 2007. *Ann Oncol* 2007;**18**:1133–44.
16. Wolmark N, Wang J, Mamounas E, Bryant J, Fisher B. Preoperative chemotherapy in patients with operable breast cancer: nine-year results from National Surgical Adjuvant Breast and Bowel Project B-18. *J Natl Cancer Inst Monogr* 2001;**30**:96–102.
17. Mauri D, Pavlidis N, Ioannidis JP. Neoadjuvant versus adjuvant systemic treatment in breast cancer: a meta-analysis. *J Natl Cancer Inst* 2005;**97**:188–94.
18. Saverio SD, Gutierrez J, Avisar E. A retrospective review with long term follow up 11,400 cases of pure mucinous breast carcinoma. *Breast Cancer Res Treat* 2008;**111**:541–7.
19. Yamaguchi J, Akashi-Tanaka S, Fukutomi T, Kinoshita T, Iwamoto E, Takasugi M. A case of mucinous carcinoma of the breast that demonstrated a good pathological response to neoadjuvant chemotherapy despite a poor clinical response. *Breast Cancer* 2006;**13**:100–3.
20. Takeuchi H, Tsuji K, Ueo H, Kano T, Maehara Y. Clinicopathological feature and long-term prognosis of apocrine carcinoma of the breast in Japanese women. *Breast Cancer Res Treat* 2004;**88**:49–54.
21. Tanaka K, Imoto S, Wada N, Sakemura N, Hasebe K. Invasive apocrine carcinoma of the breast: clinicopathologic features of 57 patients. *Breast J* 2008;**14**:164–8.
22. Pezzi CM, Patel-Parekh L, Cole K, Franko J, Klimberg VS, Bland K. Characteristics and treatment of metaplastic breast cancer: analysis of 892 cases from the National Cancer Data Base. *Ann Surg Oncol* 2007;**14**:166–73.
23. Luini A, Aguilar M, Gatti G, Fasani R, Botteri E, Brito JAD, et al. Metaplastic carcinoma of the breast, an unusual disease with worse prognosis: the experience of the European Institute of Oncology and review of the literature. *Breast Cancer Res Treat* 2007;**101**:349–53.
24. Wargtoz ES, Norris HJ. Metaplastic carcinomas of the breast. I. Matrix-producing carcinoma. *Hum Path* 1989;**20**:628–35.
25. Wargtoz ES, Does PH, Norris HJ. Metaplastic carcinomas of the breast. II. Spindle cell carcinoma. *Hum Path* 1989;**20**:732–40.
26. Wargtoz ES, Norris HJ. Metaplastic carcinomas of the breast. III. Carcinosarcoma. *Cancer* 1989;**64**:1490–9.
27. Wargtoz ES, Norris HJ. Metaplastic carcinomas of the breast. IV. Squamous cell carcinoma of ductal origin. *Cancer* 1990;**65**:272–6.
28. Wargtoz ES, Norris HJ. Metaplastic carcinomas of the breast. V. Metaplastic carcinoma with osteoclastic giant cells. *Hum Path* 1990;**21**:1142–50.
29. Rayson D, Adjei AA, Suman VJ, Wold LE, Ingle JN. Metaplastic breast cancer: prognosis and response to systemic therapy. *Ann Oncol* 1999;**10**:413–9.
30. Brown-Glaberman U, Graham A, Stopeck A. A case of metaplastic carcinoma of the breast responsive to chemotherapy with ifofamide and etoposide: improved antitumor respmnse by targeting sarcomatous features. *Breast J* 2010;**16**:663–5.
31. Cocquyt VF, Blondeel PN, Depypere HT, Preat MM, Schelfhout VR, Silva OE, et al. Different responses to preoperative chemotherapy for invasive lobular and invasive ductal breast carcinoma. *Eur J Surg Oncol* 2003;**29**:361–7.
32. Wasif N, Maggard MA, Ko CY, Giulianao AE. Invasive lobular vs. ductal breast cancer; a stage-matched comparison of outcomes. *Ann Surg Oncol* 2010;**17**:1862–9.
33. Cristofanilli M, Gonzalez-Angulo A, Sneige N, Kau SW, Broglio K, Theriault RL, et al. Invasive lobular carcinoma classic type: response to primary chemotherapy and survival outcomes. *J Clin Oncol* 2005;**23**:41–8.
34. Tubiana-Hulin M, Stevers D, Lasry S, Guinebreti□re JM, Bouita L, Cohen-Solal C, et al. Response to neoadjuvant chemotherapy in lobular and ductal breast carcinomas: a retrospective study on 860 patients from one institution. *Ann Oncol* 2006;**17**:1228–33.
35. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001;**98**:10869–74.
36. Sørlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci USA* 2003;**100**:8418–23.
37. Guarneri V, Broglio K, Kau SW, Cristofanilli M, Buzdar AU, Valero V, et al. Prognostic value of pathologic complete response after primary chemotherapy in relation to hormone receptor status and other factors. *J Clin Oncol* 2006;**24**:1037–44.
38. Kuerer HM, Newman LA, Smith TL, Ames FC, Hunt KK, Dhingra K, et al. Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. *J Clin Oncol* 1999;**17**:460–9.
39. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005;**365**:1687–717.
40. Yerushalmi R, Hayes MM, Gelmon KA. Breast carcinomas-rare types: review of the literature. *Ann Oncol* 2009;**20**:1763–70.
41. Reis-Filho JS, Milanezi F, Steele D, Savage K, Simpson PT, Nesland JM, et al. Metaplastic breast carcinomas are basal-like tumors. *Histopathology* 2006;**49**:10–21.
42. Straver ME, Glas AM, Hannemann J, Wesseling J, van de Vijver MJ Th, Rutgers EJ, et al. The 70-gene signature as as response predictor for neoadjuvant chemotherapy in breast cancer. *Breast Cancer Res Treat* 2010;**119**:551–8.
43. Gianni L, Zambetti M, Clark K, Barker J, Cronin M, Wu J, et al. Gene expression profiles in paraffin-embedded core biopsy tissue predict response to chemotherapy in women with locally advanced breast cancer. *J Clin Oncol* 2005;**23**:7265–77.
44. Tanaka-Akashi S, Shimizu C, Ando M, Shibata T, Katsumata N, Kouno T, et al. 21-Gene expression profile assay on core needle biopsies predicts responses to neoadjuvant endocrine therapy in breast cancer patients. *Breast* 2009;**18**:171–4.
45. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ, et al. Strategies for subtypes-dealing with the diversity of breast cancer: highlights of the St Gallen international expert consensus on the primary therapy of early breast cancer 2011. *Ann Oncol* 2011;**22**:1736–47.

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Efficacy of everolimus, a novel mTOR inhibitor, against basal-like triple-negative breast cancer cells

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Patients with triple-negative breast cancers (TNBCs) typically have a poor prognosis because such cancers have no effective therapeutic targets, such as estrogen receptors for endocrine therapy or human epidermal growth factor receptor 2 (HER2) receptors for anti-HER2 therapy. As the phosphatidylinositol 3′ kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) cascade is activated in TNBCs, mTOR is a potential molecular target for anticancer therapy. In this study, we investigated the antitumor activities of everolimus, an oral mTOR inhibitor, in nine TNBC cell lines. Everolimus effectively inhibited cell growth at concentrations under 100 nM (IC_{50}) in five cell lines and even in the 1-nM range in three of the five cell lines. To identify specific characteristics that could be used as predictive markers of efficacy, we evaluated the expressions of proteins in the mTOR cascade, basal markers, and cancer stem cell markers using western blotting, fluorescent *in situ* hybridization (FISH), or immunohistochemistry. All five of the sensitive cell lines were categorized as a basal-like subtype positive for either epidermal growth factor receptor (EGFR) or CK5/6, although resistant cell lines were not of this subtype and tended to exhibit the characteristics of cancer stem cells, with decreased E-cadherin and the increased expression of Snail or Twist. *In vivo* assays demonstrated antitumor activity in a mouse xenograft model of basal-like breast cancer, rather than non-basal breast cancer. These results suggest that everolimus has favorable activity against basal-like subtypes of TNBCs. Epidermal growth factor receptor and CK5/6 are positive predictive markers of the TNBC response to everolimus, while cancer stem cell markers are negative predictive markers. (*Cancer Sci* 2012; 103: 1665–1671)

Triple-negative breast cancers (TNBCs) are defined as estrogen receptor (ER)-negative, progesterone receptor (PGR)-negative, and human epidermal growth factor receptor 2 (HER2)-negative tumors; these tumors account for 11–23% of all breast cancers.[1–3] Triple-negative breast cancers follow a more aggressive clinical course than other forms of breast cancers and have a poor prognosis.[1] As TNBCs have no indications for endocrine therapy or HER2 inhibitors, which are the main treatment options for breast cancers, novel molecular-targeted therapies against TNBCs are crucially needed.

Triple-negative breast cancers are a heterogeneous population containing a subgroup that is extremely sensitive to chemotherapy, while another subgroup is resistant to such therapy.[2,4–6] For example, familial breast cancers with the *BRCA1/2* germline mutation are frequently included in the TNBC category that is chemosensitive.[5] In contrast, metaplastic carcinoma of the breast, which often lacks ER, PGR, and HER2 expressions, is quite chemoresistant.[6] Gene expression profiling can be used to separate breast cancers into five distinct molecular subtypes: luminal A (ER or PGR positive and HER2 negative); luminal B (ER or PGR positive and HER2 positive); HER2 overexpressing (ER or PGR negative and HER2 positive); normal breast-like; and basal-like.[7–10] Recently, Herschkowitz *et al.*[11] reported a novel subgroup of TNBCs – the claudin-low subgroup, which is characterized by low gene expressions of the tight junction proteins claudin 3, 4, 7, and E-cadherin – which is clearly different from the basal-like subtype. The claudin-low subtype has been shown to have cancer-stem-cell-like features because it exhibits a high CD44/CD24 expression ratio[12] and the upregulation of *snail* and *twist*,[13] which have been described as specific markers for cancer stem cells.[14,15] Clearly, TNBC subtypes must be classified to facilitate the development of effective therapies for individuals and to improve therapeutic outcomes.

Everolimus (RAD001) is an inhibitor of serine-threonine kinase mammalian target of rapamycin (mTOR) and has shown broad antitumor activities in preclinical models.[16,17] Everolimus has been approved for the treatment of refractory renal cell carcinoma,[18] progressive neuroendocrine tumors of pancreatic origin (PNET),[19] and subependymal Giant cell astrocytoma associated with tuberous sclerosis.[20] In addition, several clinical trials have reported the effectiveness of everolimus used in combination with trastuzumab or hormone therapy against HER2-overexpressing or hormone-receptor-overexpressing breast cancers, respectively.[21,22] However, the effect of everolimus against TNBCs has not yet been examined. The loss of function of phosphatase and tensin homolog deleted in chromosome 10 (PTEN) has been reported with varying frequencies in breast cancers[23,24] and has been shown to occur frequently in TNBCs.[23,25] As PTEN dysfunction leads to the activation of the phosphatidylinositol 3′ kinase (PI3K)/Akt/mTOR signaling pathway, mTOR is a potential molecular target for the treatment of TNBCs.

In this study, we investigated the antitumor activities of everolimus in TNBC cell lines *in vitro* and *in vivo* and identified predictive markers of the response of TNBCs to everolimus.

Material and Methods

Cell lines and reagents. The following TNBC cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA) for use in this study: MDA-MB-157, MDA-MB-231, MDA-MB-436, MDA-MB-468, Hs578T, BT20, BT549, HCC38, and HCC1937. All the cell lines were cultured in modified Eagle's medium essential (MEME) or RPMI medium supplemented with 10% FBS at 37°C and in humidified 5% CO_2. Everolimus was a generous gift of Novartis Pharma AG (Basel, Switzerland). GDC0914 bismesylate and

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