Article type: Original article

Prognostic significance of pathologic complete response and Ki67 expression after neoadjuvant chemotherapy in breast cancer

Tatsuya Yoshioka[1, 2], Mitsuchika Hosoda[1], Mitsugu Yamamoto[1], Kazunori Taguchi[1], Kanako C. Hatanaka[3], Emi Takakuwa[3,4], Yutaka Hatanaka[3,4], Yoshihiro Matsuno[3,4], Hiroko Yamashita[1, *]

[1]Breast and Endocrine Surgery, Hokkaido University Hospital, Kita 14 Nishi 5, Kita-ku, Sapporo 060-8648, Japan

[2]Department of Gastroenterological Surgery II, Hokkaido University Graduate School of Medicine, Kita 15 Nishi 7, Kita-ku, Sapporo 060-8638, Japan

[3]Department of Surgical Pathology, Hokkaido University Hospital, Kita 14 Nishi 5, Kita-ku, Sapporo 060-8648, Japan

[4]Research Division of Companion Diagnostics, Hokkaido University Hospital, Kita 14 Nishi 5, Kita-ku, Sapporo 060-8648, Japan

***Corresponding author:** Hiroko Yamashita

Mailing address: Breast and Endocrine Surgery, Hokkaido University Hospital, Kita 14, Nishi 5, Kita-ku, Sapporo 060-8648, Japan

Tel: +81-11-706-7381

Fax: +81-11-706-7384

E-mail: hirokoy@huhp.hokudai.ac.jp

Key words: breast cancer, neoadjuvant chemotherapy, pathologic complete response, Ki67

Abstract

Background: Recent studies have indicated that response to chemotherapy and the prognostic impact of a pathologic complete response (pCR) after neoadjuvant chemotherapy differ among breast cancer subtypes.

Methods: Women with Stage I to III breast cancer treated with anthracycline and taxane-based neoadjuvant chemotherapy (four cycles of docetaxel every 3 weeks followed by four cycles of FEC every 3 weeks) between 2006 and 2011 were retrospectively analyzed. Trastuzumab was concurrently added to docetaxel for HER2-positive breast cancer. Expression of estrogen receptor (ER), progesterone receptor (PgR), HER2, and Ki67 was examined by immunohistochemistry in pre- and post-treatment specimens. Predictive factors for neoadjuvant chemotherapy and prognosis were analyzed by breast cancer subtype.

Results: Of 64 patients, 30 (47%) were ER-positive (ER+) HER2-negative (HER2-), including 8 as luminal A (Ki67 labeling index (LI) < 14%) and 22 as luminal B (Ki67 LI ≥ 14%) subtypes, 11 (17%) were ER+ HER2-positive (HER2+), 12 (19%) were ER-negative (ER-) HER2+, and 11 (17%) were ER- HER2-. The clinical response rates were significantly higher in luminal B, ER+ HER2+, and ER- HER2+ subtypes compared with luminal A subtype. Patients whose tumors contained high Ki67 expression effectively responded to neoadjuvant chemotherapy. Ki67 LI was a predictive marker for pCR, and all patients whose tumors achieved pCR are currently disease-free. Furthermore, high

Ki67 expression in post-treatment tumors was strongly correlated with poor disease-free and overall survival regardless of subtype.

Conclusions: It is necessary to establish additional strategies to improve survival for patients whose residual tumors show high Ki67 expression after neoadjuvant chemotherapy.

Abbreviations

ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor type 2; ER+, estrogen receptor-positive; ER-, estrogen receptor-negative; HER2+, HER2-positive; HER2-, HER2-negative; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; IHC, immunohistochemistry; Ki67 LI, Ki67 labeling index; pCR, pathologic complete response; cCR, clinical complete response

Introduction

Neoadjuvant chemotherapy has been established as a standard treatment strategy for patients with not only local advanced but also operable breast cancer. Generally, any patient who is a candidate for adjuvant chemotherapy can be considered for neoadjuvant chemotherapy [1, 2]. The main expected benefit from neoadjuvant chemotherapy is reduction of the extent of surgery. The largest benefit is realized in those patients who are most likely to achieve a pathologic complete response (pCR), such as triple-negative, high-grade estrogen receptor-positive (ER+), and HER2-positive (HER2+) breast cancer [3, 4]. A recent study indicated that pCR defined as no invasive and no in situ residuals in breast and nodes could best discriminate between patients with favorable and unfavorable outcomes, and that pCR was a suitable surrogate prognostic factor for patients with luminal B, ER-negative (ER-) HER2+, and triple-negative disease but not ER+ HER2+ or luminal A tumors [5]. Thus, comparisons of pCR rates between independent trials are not sufficiently reliable to establish superior regimens because subtle differences in patient characteristics, such as ER, HER2, and grade, can have substantial impact. Unfortunately, extensive subsets of patients do not experience pCR, even with modern regimens [6, 7]. Long-term survival of patients with residual tumor largely depends on its extent and baseline prognosis [8]. Thus, observed differences in pCR have to be validated by long-term outcome in order to change clinical practice.

Ki67 is a nuclear protein that is expressed during all phases of the cell cycle

except the G0 phase, and is a marker for tumor proliferation [9]. Recent studies have shown that the so called “Luminal A” subtype—characterized by low histological grade, low proliferation as measured by Ki67, high hormone receptor status, and negative HER2 status—was less responsive to chemotherapy, and that no preferable chemotherapy regimen could be defined for treatment of this subtype [2, 10]. The potential usefulness of Ki67 in predicting response and long-term outcome has been explored by assessing pre- and post-treatment levels of Ki67 expression in neoadjuvant chemotherapy studies [11-14]. There is a general theory that biomarkers for rate of proliferation can predict responsiveness to systemic therapy, with highly proliferative tumors being more chemotherapy responsive. Thus, Ki67 might have a valuable role in predicting benefit from specific treatments in certain subtypes of breast cancer.

In this study, we retrospectively examined expression of ER, progesterone receptor (PgR), HER2, and Ki67 by immunohistochemistry in pre- and post-treatment specimens in patients with Stage I to III breast cancer who were treated with anthracycline and taxane-containing neoadjuvant chemotherapy. Predictive factors for response to neoadjuvant chemotherapy and prognosis were analyzed by breast cancer subtype.

Patients and methods

Patients and treatment

A total of 64 women with Stage I to III breast cancer treated with anthracycline and taxane-based neoadjuvant chemotherapy between 2006 and 2011 at Hokkaido University Hospital were retrospectively recruited to this study (Table 1). Patients were considered evaluable if they had completed neoadjuvant chemotherapy, and patients who had not completed all regimens were excluded. Neoadjuvant chemotherapy regimens comprosed four cycles of docetaxel 75 mg/m^2 every 3 weeks followed by four cycles of FEC (5-fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2, and cyclophosphamide 500 mg/m^2, every 3 weeks). Trastuzumab was concurrently added to docetaxel for 20 patients with HER2+ breast cancer. Clinical measurements of tumor size and nodal status were performed monthly, and the final clinical, sonographic or MRI measurements were performed 6 months after the start of treatment prior to the planned surgical excision of the tumor. Clinical responses were defined as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD) according to the Response Evaluation Criteria in Solid Tumors (RECIST, 2000). Pretreatment specimens were taken by core needle biopsies. Post-treatment specimens were obtained during surgery. The pathological response was assessed as grades 1 to 3 according to the following criteria: 0 (no response), 1 (mild to moderate response), 2 (marked response), 3 (complete response) according to the histopathological criteria for

assessment of therapeutic response in breast cancer by the Japanese Breast Cancer Society [15]. pCR was defined as no invasive and no in situ residuals in breast and nodes [5]. The median follow-up period was 32 months (range, 7 to 67 months). The study protocol was approved by the institutional review boards and conformed with the guidelines of the 1996 Declaration of Helsinki.

Immunohistochemical (IHC) analysis

One 4-μm section of each submitted paraffin block was stained first with hematoxylin-eosin to verify that an adequate number of carcinoma cells were present and that the fixation quality was adequate for IHC analysis. Serial sections (4μm) were prepared from selected blocks and float-mounted on adhesive-coated glass slides for IHC and fluorescence *in situ* hybridization (FISH) [16]. IHC status of ER, PgR, and HER2 was determined using the PATHWAY rabbit monoclonal antibodies (clone SP1, 1E2, and 4B5, respectively) and iView DAB Detection Kit (Ventana Medical Systems, Inc., Tucson, AZ, USA). The expression of ER and PgR was estimated by staining of cell nuclei, and was considered positive when the percentage of positive cells was at least 1% [17]. To determine the level of HER2 expression, the membrane staining pattern was estimated and scored on a scale of 0 to 3+. Tumors with a score of 2+ were tested for gene amplification by FISH using the PathVysion assay (Vysis, Abbott Laboratories, Abbott Park, IL, USA). A ratio of HER2 gene/chromosome 17 >2.2 was considered positive.

Tumors were considered HER2-positive if IHC staining was 3+ or FISH positive [18]. IHC for Ki67 was performed with mouse monoclonal anti-human Ki67 antibody (MIB-1, Dako, Glostrup, Denmark) at 1:200 dilution and the Dako FLEX Envision system for visualization. The labeling index (LI) was assessed as the percentage of tumor cells showing definite nuclear staining among >1000 invasive tumor cells [19] using NanoZoomer 2.0-HT (Hamamatsu photonics, Hamamatsu, Japan) for slide scanning and Tissue Studio (Definiens, Munich, Germany) for automated scoring.

Statistical analysis

The chi-square test was used to compare breast cancer subtypes with clinical and pathological response to neoadjuvant chemotherapy. The unpaired *t*-test was used to compare Ki67 LI between pCR and non pCR. Spearman's rank correlation test was used to study the relationship between tumor grade and Ki67 LI. Cox's proportional hazards model was used for univariate and multivariate analyses of predictive values for neoadjuvant chemotherapy and prognostic values for disease-free and overall survival. Estimation of survival was performed using the Kaplan-Meier method, and differences between survival curves were assessed with the log-rank test.

Results

Breast cancer subtypes affect clinical response to neoadjuvant chemotherapy

Of the 64 patients in the study, 30 (47%) were ER+ HER2-, 11 (17%) were ER+ HER2+, 12 (19%) were ER- HER2+, and 11 (17%) were ER- HER2- (Table 1). In patients with ER+ HER2- disease, 8 were classified as luminal A (ER+ HER2- and Ki67 LI < 14%) and 22 were classified as luminal B (ER+ HER2- and Ki67 LI ≥ 14%) subtypes. The breast cancer subtypes defined by ER, HER2, and Ki67 were significantly associated with clinical response to neoadjuvant chemotherapy ($p = 0.035$, Table 2). The clinical response rates (PR + CR) were significantly higher in luminal B (73%), ER+ HER2+ (100%), and ER- HER2+ (75%) subtypes compared with luminal A subtype (25%). The clinical response rate in ER- HER2- subtype was 64%. Although pCR was not significantly associated with breast cancer subtypes, the pCR rate in the ER- HER2+ subtype was the highest (33%) among all subtypes (Table 3). No patient achieved pCR in the luminal A subtype, and 9% of patients achieved pCR in luminal B, ER+ HER2+, and ER- HER2- subtypes.

Ki67 expression is a predictive factor for pCR in neoadjuvant chemotherapy

We next examined whether Ki67 LI affected the pathological response to neoadjuvant chemotherapy. When analyzed by breast cancer subtype, tumors with high Ki67 LI showed significantly improved pCR rates in the luminal B subtype ($p = 0.035$),

whereas no association was observed between Ki67 LI and pCR rates in HER2-positive disease (Table 4). Tumors with high Ki67 LI also achieved pCR in the triple-negative subtype, although only one in eleven patients showed pCR in this subtype.

To analyze factors that predict pCR, clinicopathological factors including Ki67 LI were examined for pCR association by univariate and multivariate analyses (Table 5). Tumor grade and Ki67 LI were significantly associated with pCR by univariate analysis ($p = 0.048$ and $p = 0.022$, respectively). Tumor grade was strongly correlated with Ki67 LI before neoadjuvant chemotherapy ($p < 0.00001$ by Spearman's rank correlation test), and correlation between Ki67 LI and pCR ($p = 0.022$) was greater than that between tumor grade and pCR ($p = 0.048$), suggesting that the Ki67 LI value before neoadjuvant chemotherapy is a predictive factor for pCR.

Ki67 expression after neoadjuvant chemotherapy is an independent prognostic factor for survival

We then analyzed factors that affected the prognosis for patients who received neoadjuvant chemotherapy. Clinicopathological factors and expression of ER, PgR, HER2, and Ki67 in tumors both in pre- and post-treatment specimens were estimated. Lymph node status, grade after neoadjuvant chemotherapy (post grade), and Ki67 LI after neoadjuvant chemotherapy (post Ki67 LI) were significantly associated with disease-free survival by univariate analysis ($p = 0.02$, $p = 0.02$, and $p = 0.003$, respectively, Table 6).

Post grade and post Ki67 LI were strongly correlated (p = 0.0023 by Spearman's rank correlation test), and correlation between post Ki67 LI and disease-free survival (p = 0.003) was greater than that between post grade and disease-free survival (p = 0.02). Therefore, lymph node status and post Ki67 LI were selected for multivariate analysis. Post Ki67 LI was the only factor that was significantly associated with disease-free survival by multivariate analysis (p = 0.005), suggesting that Ki67 LI after neoadjuvant chemotherapy is a prognostic factor for disease-free survival.

A Kaplan-Meier analysis showed that low Ki67 LI (<40%) after neoadjuvant chemotherapy was strongly associated with increased disease-free and overall survival (p = 0.0004 and p = 0.0003, respectively; Fig. 1A and 1B).

All patients whose tumors achieved pCR in this analysis are currently disease-free. A Kaplan-Meier analysis showed that pCR was correlated with improved disease-free and overall survival, although a statistical analysis was not applicable (Fig. 2A and 2B).

Discussion

We analyzed expression of ER, PgR, HER2, and Ki67 in pre- and post-treatment samples in order to investigate their prognostic and predictive potential in women with Stage I to III breast cancer who had been treated with anthracycline and taxane-containing neoadjuvant chemotherapy. A high Ki67 expression in pre-treatment tumors was significantly associated with improved pCR rates, and a high Ki67 expression in post-treatment tumors was strongly correlated with poor disease-free and overall survival regardless of subtype.

Recent analyses have shown that the prognostic value of pCR after neoadjuvant chemotherapy has to be rated differently according to subtype [3]. Whereas patients with triple-negative, ER- HER2+, and luminal B tumors with a pCR after neoadjuvant chemotherapy showed a significantly better outcome than did patients without a pCR, this prognostic impact was not seen in patients with luminal A or ER+ HER2+ tumors [5]. Although the precise definition of pCR has not been established, von Minckwits and colleagues recently reported that pCR defined as no invasive and no in situ residuals in breast and nodes was most highly correlated with the best survival [5]. Therefore, we used this definition for pCR in the present study. Our results showed that any patient with luminal A subtype did not achieve pCR, and that all patients whose tumors, including luminal B, ER+ HER2+, ER- HER2+, and ER- HER2- tumors, achieved pCR are currently disease-free.

Our present study demonstrated that Ki67 LI was a predictive marker for pCR. However, no association was observed between Ki67 LI and pCR rates in ER+ HER2+ and ER- HER2+ diseases. In contrast, the pCR rate in ER- HER2+ subtype was much higher (33%) than that in ER+ HER2+ tumors (9%). Although trastuzumab was added to neoadjuvant chemotherapy in HER2-positive breast cancer, the response to neoadjuvant chemotherapy was clearly different among these two subtypes.

In ER+ HER2- breast cancer, recent studies have indicated that chemotherapy indications follow an inverse pattern in relation to endocrine responsiveness [2]. The less endocrine-responsive a tumor—such as a tumor with low expression levels of ER and high expression levels of Ki67—the higher the indication for chemotherapy [20]. Our previous study showed that patients whose breast tumors showed low Ki67 expression displayed longer time to progression during first-line endocrine therapy with aromatase inhibitors [21]. On the other hand, randomized clinical studies on adjuvant chemotherapy have retrospectively reviewed data and reported that Ki67 was a predictor of benefit from chemotherapy with endocrine therapy in ER-positive breast cancer [22-24]. Therefore, expression levels of Ki67 are involved in biological characteristics of the tumors, including prognosis and response to certain kinds of therapies in ER-positive breast cancer.

We have shown here that the level of Ki67 LI in the residual tumors after neoadjuvant chemotherapy was predictive of survival, and that pre-treatment tumor size

and lymph node status were not predictive. Jones and colleagues also demonstrated that post-chemotherapy Ki67 level was a strong predictor of outcome for patients not achieving a pCR [12]. Patients would avoid an initial high-risk prognosis, even if they were node-positive or had a large tumor size, if they had a pCR. For patients with high Ki67 levels or ER-negative tumors in residual disease, new treatment strategies have to be found.

In conclusion, the present data indicate that patients whose tumors contained high levels of Ki67 effectively responded to anthracycline and taxane-containing neoadjuvant chemotherapy. Ki67 LI was a predictive marker for pCR, and all patients whose tumors achieved pCR were disease-free at the time of this publication. Furthermore, a high Ki67 expression in post-treatment tumors was strongly correlated with poor disease-free and overall survival regardless of subtype. It is thus necessary to establish additional strategies to improve survival for patients whose residual tumors show high Ki67 expression after neoadjuvant chemotherapy.

Conflict of interest statement

All authors have no conflict of interest to disclose.

References

1. Kaufmann M, von Minckwitz G, Mamounas EP, Cameron D, Carey LA, Cristofanilli M, et al. Recommendations from an international consensus conference on the current status and future of neoadjuvant systemic therapy in primary breast cancer. Ann Surg Oncol. 2012;19:1508-16.
2. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B, Senn HJ. 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.
3. von Minckwitz G. Neoadjuvant chemotherapy in breast cancer-insights from the German experience. Breast Cancer. 2012;19:282-8.
4. Houssami N, Macaskill P, von Minckwitz G, Marinovich ML, Mamounas E. Meta-analysis of the association of breast cancer subtype and pathologic complete response to neoadjuvant chemotherapy. Eur J Cancer. 2012;48:3342-54.
5. von Minckwitz G, Untch M, Blohmer JU, Costa SD, Eidtmann H, Fasching PA, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol. 2012;30:1796-804.
6. Jones RL, Salter J, A'Hern R, Nerurkar A, Parton M, Reis-Filho JS, et al. Relationship between oestrogen receptor status and proliferation in predicting response and long-term outcome to neoadjuvant chemotherapy for breast cancer. Breast Cancer Res Treat. 2010;119:315-23.
7. Lips EH, Mulder L, de Ronde JJ, Mandjes IA, Vincent A, Vrancken Peeters MT, et al. Neoadjuvant chemotherapy in ER+ HER2- breast cancer: response prediction based on immunohistochemical and molecular characteristics. Breast Cancer Res Treat. 2012;131:827-36.
8. Montagna E, Bagnardi V, Rotmensz N, Viale G, Pruneri G, Veronesi P, et al. Pathological complete response after preoperative systemic therapy and outcome: relevance of clinical and biologic baseline features. Breast Cancer Res Treat. 2010;124:689-99.
9. Yerushalmi R, Woods R, Ravdin PM, Hayes MM, Gelmon KA. Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol. 2010;11:174-83.
10. Esserman LJ, Berry DA, DeMichele A, Carey L, Davis SE, Buxton M, et al. Pathologic complete response predicts recurrence-free survival more effectively by cancer subset: results from the I-SPY 1 TRIAL--CALGB 150007/150012, ACRIN 6657. J Clin Oncol. 2012;30:3242-9.
11. Burcombe R, Wilson GD, Dowsett M, Khan I, Richman PI, Daley F, et al. Evaluation of Ki-67 proliferation and apoptotic index before, during and after neoadjuvant chemotherapy for primary breast cancer. Breast Cancer Res.

2006;8:R31.

12. Jones RL, Salter J, A'Hern R, Nerurkar A, Parton M, Reis-Filho JS, et al. The prognostic significance of Ki67 before and after neoadjuvant chemotherapy in breast cancer. Breast Cancer Res Treat. 2009;116:53-68.
13. Fasching PA, Heusinger K, Haeberle L, Niklos M, Hein A, Bayer CM, et al. Ki67, chemotherapy response, and prognosis in breast cancer patients receiving neoadjuvant treatment. BMC Cancer. 2011;11:486.
14. Matsubara N, Mukai H, Fujii S, Wada N. Different prognostic significance of Ki-67 change between pre- and post-neoadjuvant chemotherapy in various subtypes of breast cancer. Breast Cancer Res Treat. 2013;137:203-12.
15. 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.
16. Yamashita H, Takahashi S, Ito Y, Yamashita T, Ando Y, Toyama T, et al. Predictors of response to exemestane as primary endocrine therapy in estrogen receptor-positive breast cancer. Cancer Sci. 2009;100:2028-33.
17. 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.
18. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol. 2007;25:118-45.
19. Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, et al. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst. 2011;103:1656-64.
20. Piccart-Gebhart MJ. New developments in hormone receptor-positive disease. Oncologist. 2010;15 Suppl 5:18-28.
21. Endo Y, Toyama T, Takahashi S, Sugiura H, Yoshimoto N, Iwasa M, et al. High estrogen receptor expression and low Ki67 expression are associated with improved time to progression during first-line endocrine therapy with aromatase inhibitors in breast cancer. Int J Clin Oncol. 2011;16:512-8.
22. Viale G, Regan MM, Mastropasqua MG, Maffini F, Maiorano E, Colleoni M, et al. Predictive value of tumor Ki-67 expression in two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer. J Natl Cancer Inst. 2008;100:207-12.
23. Hugh J, Hanson J, Cheang MC, Nielsen TO, Perou CM, Dumontet C, et al. Breast cancer subtypes and response to docetaxel in node-positive breast cancer: use of an

immunohistochemical definition in the BCIRG 001 trial. J Clin Oncol. 2009;27:1168-76.

24. Penault-Llorca F, Andre F, Sagan C, Lacroix-Triki M, Denoux Y, Verriele V, et al. Ki67 expression and docetaxel efficacy in patients with estrogen receptor-positive breast cancer. J Clin Oncol. 2009;27:2809-15.

Figure legends

Figure 1: Disease-free (A) and overall (B) survival according to Ki67 LI after neoadjuvant chemotherapy. Patients whose residual tumors contained 40% or more Ki67-positive cells displayed shorter disease-free ($p = 0.0004$) and overall ($p = 0.0003$) survival.

Figure 2: Disease-free (A) and overall (B) survival according to pCR and non pCR after neoadjuvant chemotherapy. All patients whose tumors achieved pCR were disease-free.

Figure 1

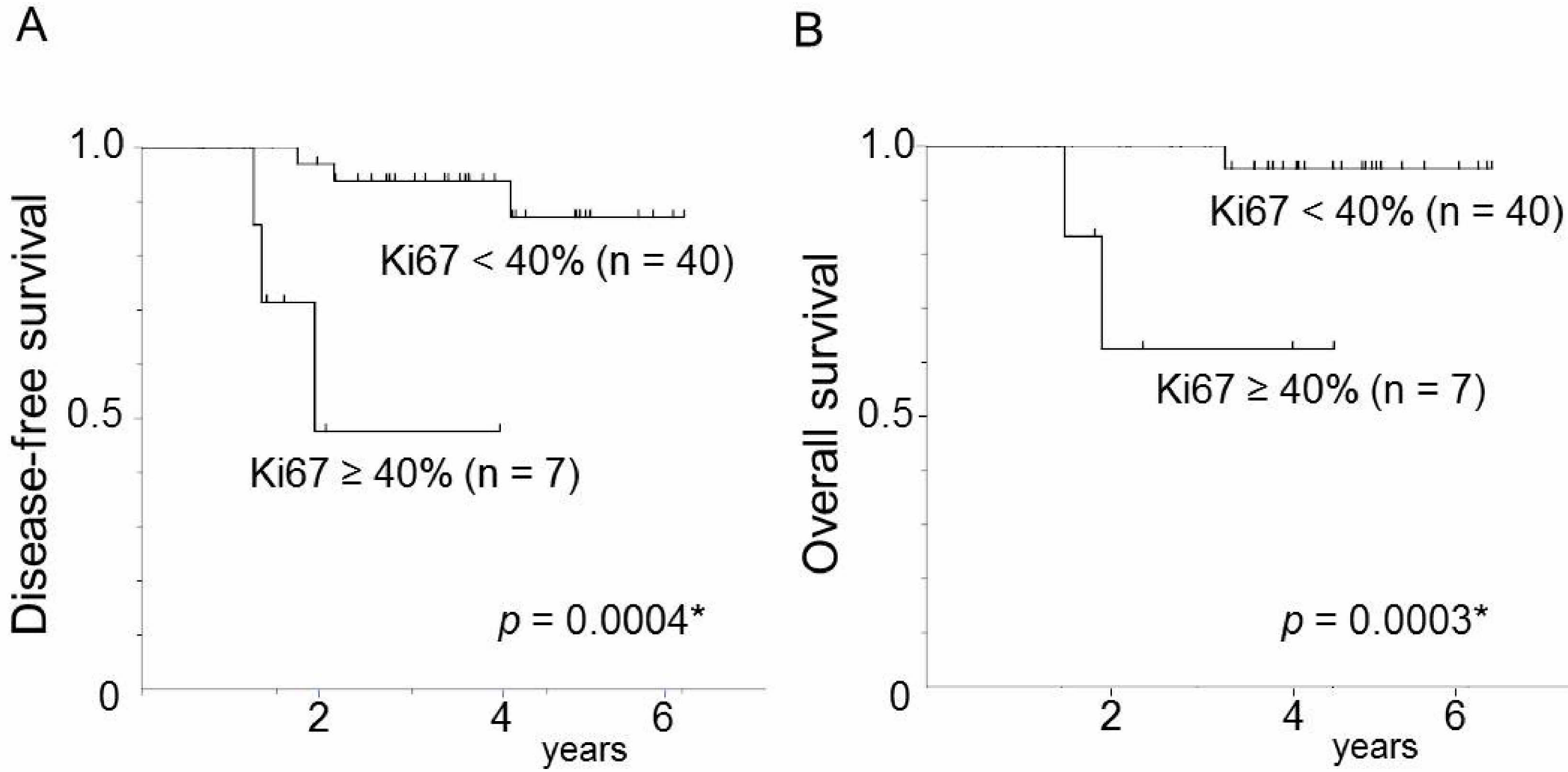
A
B
1.0
0.5
0
Disease-free survival
Overall survival
Ki67 < 40% (n = 40)
Ki67 ≥ 40% (n = 7)
p = 0.0004*
p = 0.0003*
2
4
6
years

Figure 2

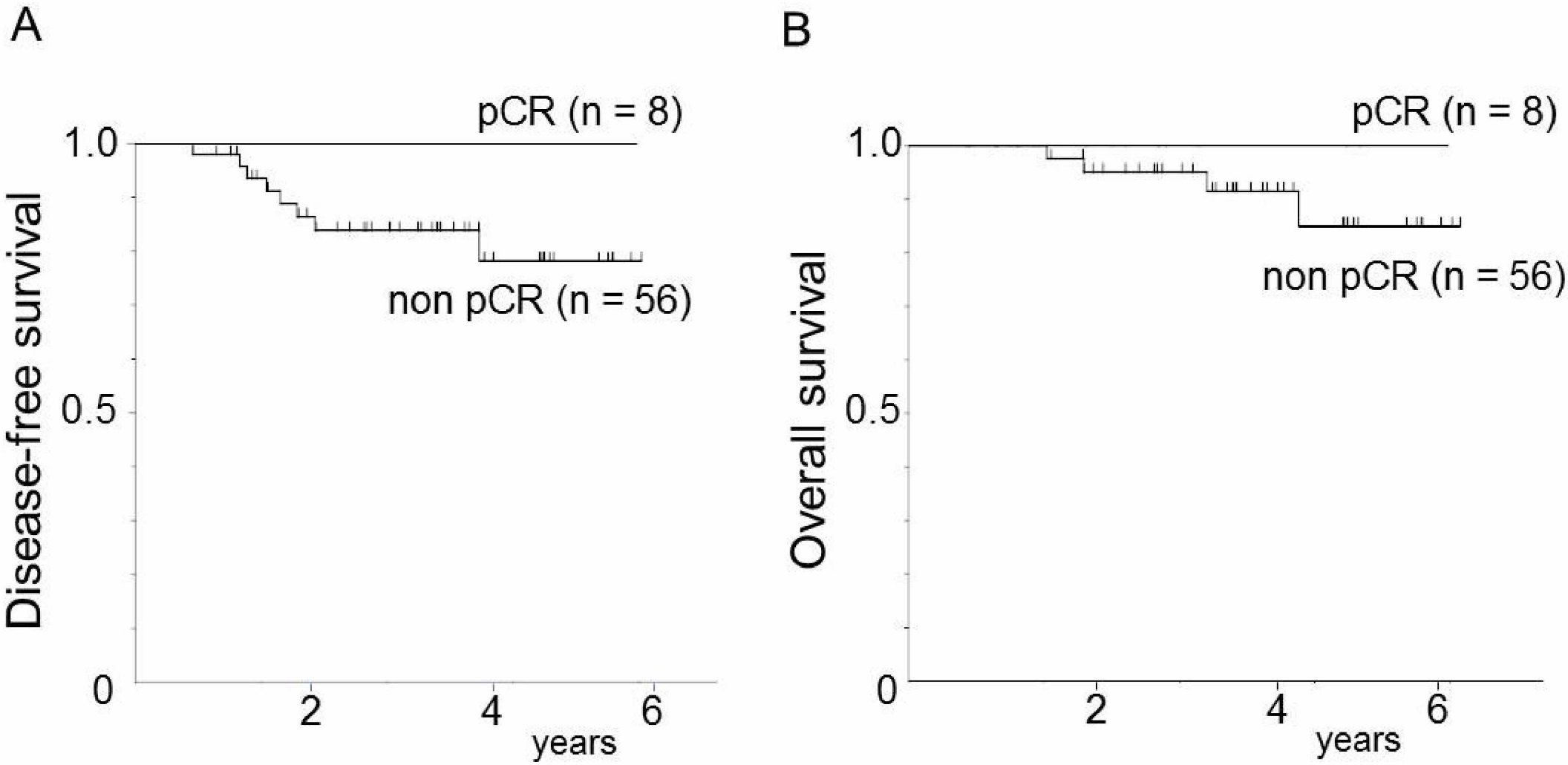
A
pCR (n = 8)
non pCR (n = 56)
Disease-free survival
1.0
0.5
0
2
4
6
years
B
pCR (n = 8)
non pCR (n = 56)
Overall survival
1.0
0.5
0
2
4
6
years

Table 1. Clinicopathological characteristics of patients and breast tumors

Factor		No. (%)
All		64 (100%)
Age, years (median, range)	52 (28-71)	
Tumor stage		
T1		12 (19%)
T2		40 (63%)
T3		12 (19%)
N stage		
N0		25 (39%)
N1		27 (42%)
N2		8 (13%)
N3		4 (6%)
Tumor grade		
1		10 (16%)
2		24 (37%)
3		30 (47%)
ER, HER2 subtype		
ER+ HER2-	luminal A	8 (13%)
	luminal B	22 (34%)
ER+ HER2+		11 (17%)
ER- HER2+		12 (19%)
ER- HER2-		11 (17%)
Ki67 status (LI)		
< 14%		9 (14%)
14% ≤ LI < 40%		34 (53%)
≥ 40%		21 (33%)
Neoadjuvant treatment		
FEC - docetaxel		44 (69%)
FEC - docetaxel + trastuzumab		20 (31%)
Surgery type		
Mastectomy		21 (33%)
Lumpectomy		43 (67%)
Postoperative adjuvant therapy		
Any endocrine therapy		40 (63%)
Tamoxifen		25 (39%)
Aromatase inhibitor		17 (27%)
Ovarian suppression		4 (6%)
Trastuzumab		21 (33%)

Table 2. Clinical response by breast cancer subtype

Subtype		No.	SD	PR	CR
ER+ HER2-	luminal A	8	6 (75%)	2 (25%)	0 (0%)
	luminal B	22	6 (27%)	13 (59%)	3 (14%)
ER+ HER2+		11	0 (0%)	7 (64%)	4 (36%)
ER- HER2+		12	3 (25%)	5 (42%)	4 (33%)
ER- HER2-		11	4 (36%)	6 (55%)	1 (9%)

Table 3. pCR and non pCR rates after neoadjuvant chemotherapy by breast cancer subtype

Subtype		No.	pCR (%)	non pCR (%)
ER+ HER2-	luminal A	8	0 (0%)	8 (100%)
	luminal B	22	2 (9%)	20 (91%)
ER+ HER2+		11	1 (9%)	10 (91%)
ER- HER2+		12	4 (33%)	8 (67%)
ER- HER2-		11	1 (9%)	10 (91%)

Table 4. Ki67 LI before neoadjuvant chemotherapy by breast cancer subtype according to pathological response

Subtype		No.	non pCR mean ± SD	pCR mean ± SD	*p*
All			33.3% ± 17.3%	50.7% ± 21.5%	0.012*
ER+ HER2-	luminal A	8	7.6% ± 4.1%	not applicable	
	luminal B	22	32.0% ± 12.8%	58.8% ± 44.3%	0.035*
ER+ HER2+		11	44.2% ± 15.3%	48.2% ± 0%	
ER- HER2+		12	40.3% ± 14.2%	40.8% ± 4.3%	0.957
ER- HER2-		11	41.0% ± 16.7%	76.8% ± 0%	

* $p < 0.05$ is considered significant.

Table 5. Univariate and multivariate analyses of factors predictive for pCR

variables	univariate analysis			multivariate analysis		
	RR	95%CI	*p*	RR	95% CI	*p*
Age	1.162	0.252 - 5.349	0.85			
T	0.504	0.144 - 0.458	0.28			
N	0.681	0.247 - 1.878	0.46			
Tumor grade	7.871	1.061 - 61.01	0.048*			
ER	0.284	0.061 - 1.322	0.11			
PgR	0.333	0.062 - 1.79	0.20			
HER2	3.519	0.756 - 16.36	0.11			
Ki67 LI	167.3	2.068 - 13527	0.022*	167.3	2.068 - 13527	0.022*

RR, relative risk; CI, confidence interval

* $p < 0.05$ is considered significant.

Table 6. Univariate and multivariate analyses of factors predictive for disease-free survival

variables	univariate analysis			multivariate analysis		
	RR	95%CI	*p*	RR	95% CI	*p*
Age	1.056	0.961 - 1.160	0.26			
T	1.075	0.340 - 3.391	0.90			
N	2.653	1.170 - 6.018	0.02*	2.564	0.944 - 6.962	0.065
pre grade	1.947	0.587 - 6.464	0.28			
post grade	4.722	1.265 - 17.62	0.02*			
pre ER	0.240	0.033 - 1.758	0.16			
post ER	0.092	0.008 - 1.098	0.06			
pre PgR	0.010	0.000 - 9.730	0.19			
post PgR	0.162	0.004 - 6.408	0.33			
HER2	0.761	0.147 - 3.929	0.74			
pre Ki67 LI	7.894	0.214 - 291.0	0.26			
post Ki67 LI	69.23	4.252 - 1127	0.003*	75.75	3.597 - 1595	0.005*
cCR	0.642	0.212 - 1.942	0.43			
pCR	1.027	0.626 - 1.685	0.92			

RR, relative risk; CI, confidence interval

* $p < 0.05$ is considered significant.