Significance of intrinsic breast cancer subtypes on the long-term prognosis after neoadjuvant chemotherapy

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Abstract

Background: The prognosis of breast cancer and the treatment response to neoadjuvant chemotherapy (NAC) differ depending on the intrinsic molecular subtypes. We evaluated the prognostic significance of immunohistological subtypes in patients with recurrent breast cancer after treatment with NAC and surgery.

Methods: A total of 237 patients with breast cancer treated with NAC and subsequent curative surgery between 2007 and 2015 were analyzed. The correlation between intrinsic molecular subtypes and clinicopathological features, prognosis, and pathological complete response (pCR) rate of NAC were investigated retrospectively.

Results: There were 55 (23.2%) patients with recurrence after surgery. No significant difference in post-recurrence survival (PRS) was noted among the subtypes (p = 0.397). In patients with estrogen receptor-positive human epidermal growth factor receptor (HER) 2-negative (luminal) malignancy, PRS was significantly better in the pCR group than in the non-pCR group (p = 0.031). Conversely, pCR was not a significant predictor of improved PRS in patients with triple-negative breast cancer (TNBC; p = 0.329). Multivariate analysis revealed that the efficacy of NAC [hazard ratio (HR) 300.204, p < 0.001] and the initial metastasis site (HR 15.037, p = 0.005) were independent predictors for PRS in patients with luminal breast cancer, while Ki-67 (HR 51.171, p = 0.020) and the initial metastasis site (HR 13.318, p = 0.048) were independent predictors for PRS in patients with TNBC.

Conclusions: The prognostic factors for each intrinsic subtype should be evaluated separately in patients with recurrent breast cancer following NAC and surgery.

Keywords: Breast cancer, Neoadjuvant chemotherapy, Post-recurrence survival, Intrinsic subtype, Long-term prognosis

Background

Breast cancer is a heterogeneous disease. Based on gene expression profiling derived from complementary deoxyribonucleic acid (cDNA) microarrays, breast cancer is classified into distinct molecular subtypes, and this diversity is clinically useful in obtaining prognostic information [1].

Treatment with neoadjuvant chemotherapy (NAC) increases the rate of breast-conserving surgery and reduces the risk of postoperative recurrence in patients with resectable breast cancer [2, 3]. In particular, pathological complete response (pCR) after NAC is an independent predictor of favorable outcome in human epidermal growth factor receptor (HER) 2-enriched and triple-negative breast cancer (TNBC) subtypes [4–9]. Although recurrence and metastasis remain the major problems in curative treatment [10], some patients with recurrent breast cancer have a relatively good post-recurrence survival (PRS). PRS is primarily related to tumor biology, including intrinsic subtypes.
However, few studies have examined the relationship between intrinsic subtypes and the PRS of patients with recurrent breast cancer [11–14]. This study aimed to evaluate the clinicopathological features and PRS according to intrinsic molecular subtypes of breast cancer in patients treated with NAC and subsequent curative surgery. To our knowledge, this study is the first to demonstrate the prognostic significance of immunohistological subtypes in patients with recurrent breast cancer after treatment with NAC and surgery.

**Methods**

**Patients**

This study included 237 patients with resectable, early-stage breast cancer diagnosed as stage II A (T1, N1, M0 or T2, N0, and M0), IIB (T2, N1, M0 or T3, N0, and M0), or II IA (T1-2, N2, M0 or T3, N1-2, and M0) treated with NAC between 2007 and 2015. Tumor stage and T and N factors were stratified based on the TNM Classification of Malignant Tumors, UICC Seventh Edition [15]. Breast cancer was confirmed histologically via core needle biopsy, or ultrasonography-guided vacuum-assisted biopsy. The tumor stage was determined via systemic imaging studies using computed tomography and bone scintigraphy. Tumors were classified into intrinsic breast cancer subtypes according to the immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PgR), HER-2, and Ki-67. The cut-offs for ER and PgR positivity were both $> 0\%$ positive tumor cells with nuclear staining. Tumors with (1) $3+$ HER2 on immunohistochemical staining, (2) HER2/centromere 17 ratio of $\geq 2.0$ [16, 17], and a (3) a Ki-67 labeling index of $\geq 14\%$ tumor cells on nuclear staining, were considered to exhibit HER2 overexpression [18]. Meanwhile, tumors with a $2+$ HER2 on immunohistochemical staining were analyzed further via fluorescence in situ hybridization.

All patients received a standard NAC protocol consisting of four courses of FEC 100 (500 mg/m$^2$ fluorouracil, 100 mg/m$^2$ epirubicin, and 500 mg/m$^2$ cyclophosphamide) every 3 weeks, followed by 12 courses of 80 mg/m$^2$ paclitaxel administered weekly [19, 20]. Sixty-four patients had HER2-positive breast cancer and were administered additional [weekly (2 mg/kg) or tri-weekly (6 mg/kg)] trastuzumab during the paclitaxel treatment [21]. Chemotherapy was administered in the outpatient department. Therapeutic anti-tumor effects were assessed according to the Response Evaluation Criteria in Solid Tumors [22]. pCR was defined as the complete disappearance of the invasive compartment of the lesion with or without intraductal components, including the lymph nodes [2]. Patients underwent mastectomy or breast-conserving surgery after NAC. All patients who underwent breast-conserving surgery also underwent postoperative radiotherapy of the remnant breast. Relapse-free survival (RFS) was defined as the absence of all local, loco-regional, and distant recurrences. Conversely, PRS was defined as the time from tumor relapse to death from any cause. All patients were followed up via physical examination every 3 months, with ultrasonography every 6 months, and computed tomography and bone scintigraphy annually.

**Ethics statement**

This study was conducted at Osaka City University Graduate School of Medicine, Osaka, Japan, according to the Reporting Recommendations for Tumor Marker Prognostic Studies guidelines and following a retrospectively written research, pathological evaluation, and statistical plan [23]. The study protocol was approved by the Ethics Committee of Osaka City University. Written informed consent was obtained from all subjects (#926).

**Statistical analyses**

Statistical analyses was performed using the JMP13 software program (SAS Institute, Cary, NC, USA). The associations between intrinsic breast cancer subtypes and clinicopathological variables were evaluated using the $\chi^2$ test (or Fisher’s exact test when necessary). The Kaplan–Meier method was used to estimate RFS and PRS. The association between breast cancer subtypes and survival was analyzed via Kaplan–Meier plots and log-rank testing. The Cox proportional hazards model was used to compute univariate and multivariate hazards ratios (HR) for the study parameters with 95% confidence interval (CI). A p value of $< 0.05$ was considered significant.

**Results**

**Analyses of all breast cancer patients**

The correlation between clinicopathological features and each intrinsic subtype is presented in Table 1. A total of 237 patients were included in this study. Among these, 93 (39.2%), 21 (8.9%), 43 (18.1%), and 80 (33.8%) had estrogen receptor-positive HER2-negative (luminal), luminal-HER2, HER2-enriched, and TNBC, respectively. Evaluation based on clinicopathological features showed that the pCR rate was significantly higher in patients with HER2-enriched breast cancer and TNBC ($p=0.001$). The median follow-up period for RFS was 4.2 years (range 0.1–10.0 years). RFS was not significantly different in each subtype ($p=0.784$, log-rank) (Fig. 1a), and it was also significantly longer in patients who achieved pCR than those who did not ($p=0.018$, log-rank) (Fig. 1b). In univariate analysis, RFS exhibited a significant relationship with Ki-67 (HR 0.553, 95% CI 0.325–0.945, $p=0.031$) and pathological response (HR 2.046, 95% CI 1.143–3.891, $p=0.015$). Multivariate analysis revealed
that Ki-67 (HR 0.548, 95% CI 0.300–0.991, \( p = 0.047 \)) and pathological response (HR 1.886, 95% CI: 1.005–3.803, \( p = 0.048 \)) were independent prognostic factors for recurrence (Table 2).

Additionally, we investigated the prognostic factors for RFS in each breast cancer subtype. Among the 93 patients with luminal type, no significant difference was observed in RFS according to pathological response (\( p = 0.731 \), log-rank) (Fig. 1c). In univariate analysis, no clinicopathological feature correlated significantly with RFS. Meanwhile, multivariate analysis revealed that lymph node (HR 4.842, 95% CI 1.336–16.230, \( p = 0.013 \)) and Ki-67 (HR 0.336, 95% CI 0.119–0.906, \( p = 0.031 \)) were independent prognostic factors for recurrence (Table 2). Among the 43 patients with HER2-enriched breast cancer, no significant difference was noted in RFS in relation to pathological response (\( p = 0.506 \), log-rank) (Fig. 1d). In univariate and multivariate analyses, there was no independent prognostic factor for recurrence in this study (Table 2). Among the 80 patients with TNBC, RFS was significantly longer in patients who achieved pCR than those who did not (\( p = 0.005 \), log-rank) (Fig. 1e). In univariate analysis, only pathological response (HR 4.251, 95% CI 1.557–14.857, \( p = 0.004 \)) was significantly correlated with RFS. Multivariate analysis also revealed that only pathological response (HR 5.013, 95% CI 1.612–19.386, \( p = 0.004 \)) was an independent prognostic factor for survival. Because the number of patients with luminal-HER2 breast cancer was small (\( n = 21 \)), statistical analyses were not performed (Table 2).

### Analyses of patients with recurrence after surgery

Among the 237 patients, 55 relapsed after surgery. The correlation between clinicopathological features and each intrinsic subtype is presented in Table 3. Among the 55 patients who relapsed, 23 (41.8%), 3 (5.4%), 9 (16.4%), and 20 (36.4%) had luminal, luminal-HER2, HER2-enriched, and TNBC, respectively. Evaluation based on clinicopathological features revealed that there was no significant correlation between each intrinsic subtype and any clinicopathological parameter, including pCR (\( p = 0.306 \)). The median follow-up period for PRS was 1.5 years (range 0.1–7.9 years). Although PRS was the worst in patients with TNBC, it was not significantly different in each subtype (\( p = 0.397 \), log-rank) (Fig. 2a). PRS was also significantly longer in patients who achieved pCR than those who did not (\( p = 0.021 \), log-rank) (Fig. 2b). In univariate analysis, PRS exhibited a significant relationship

### Table 1 Correlation between clinicopathological features and each intrinsic subtype in 237 patients treated with NAC

| Parameters                      | Intrinsic subtype | p-value |
|---------------------------------|-------------------|---------|
|                                | Luminal (n = 93)  |         |
| Age at operation                |                   |         |
| ≤ 56                            | 48 (51.6%)        |         |
| > 56                            | 45 (48.4%)        |         |
| Menopause                       |                   |         |
| Pre-                            | 42 (45.1%)        |         |
| Post                            | 51 (54.9%)        |         |
| Tumor size (cm)                 |                   |         |
| ≤ 2                             | 13 (14.0%)        |         |
| > 2                             | 80 (86.0%)        |         |
| Lymph node status               |                   |         |
| Negative                        | 20 (21.5%)        |         |
| Positive                        | 73 (78.5%)        |         |
| Nuclear grade                   |                   |         |
| 1, 2                            | 76 (81.7%)        |         |
| 3                               | 17 (18.3%)        |         |
| Ki67 (%)                        |                   |         |
| ≤ 14                            | 34 (36.6%)        |         |
| > 14                            | 59 (63.4%)        |         |
| Pathological response           |                   |         |
| Non-pCR                         | 70 (73.8%)        |         |
| pCR                             | 23 (26.2%)        |         |

NAC neoadjuvant chemotherapy, HER2 human epidermal growth factor receptor 2, TNBC triple-negative breast cancer, pCR pathological complete response

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| Age at operation                |                   |         |
| ≤ 56                            | 48 (51.6%)        |         |
| > 56                            | 45 (48.4%)        |         |
| Menopause                       |                   |         |
| Pre-                            | 42 (45.1%)        |         |
| Post                            | 51 (54.9%)        |         |
| Tumor size (cm)                 |                   |         |
| ≤ 2                             | 13 (14.0%)        |         |
| > 2                             | 80 (86.0%)        |         |
| Lymph node status               |                   |         |
| Negative                        | 20 (21.5%)        |         |
| Positive                        | 73 (78.5%)        |         |
| Nuclear grade                   |                   |         |
| 1, 2                            | 76 (81.7%)        |         |
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Fig. 1 Relapse-free survival (RFS) for each intrinsic subtype and for pathological response. RFS was not significantly different in each subtype (p = 0.784, log-rank) (a). Patients who achieved pCR had significantly better RFS among all breast cancers (p = 0.018, log-rank) (b). RFS was not significantly different between patients with pCR and non-pCR of Luminal breast cancer (p = 0.731, log-rank) (c). Patients who achieved pCR had significantly better RFS of HER2-enriched breast cancer (p = 0.506, log-rank) (d). Patients who achieved pCR had significantly better RFS of TNBC (p = 0.005, log-rank) (e).
with pathological response (HR 3.321, 95% CI 1.264–11.437, p = 0.013) and RFS (HR 2.439, 95% CI 1.005–7.269, p = 0.049). Multivariate analysis showed that tumor size (HR 5.533, 95% CI 1.251–39.287, p = 0.023), Ki-67 (HR 2.606, 95% CI 1.028–6.780, p = 0.044), pathological response (HR 4.355, 95% CI 1.438–16.871, p = 0.008), and metastatic site (HR 2.496, 95% CI 1.007–6.126, p = 0.048) had strong prognostic significance for PRS (Table 4).

Among the 23 patients with luminal type, PRS was significantly longer in patients who achieved pCR than those who did not (p = 0.031, log-rank) (Fig. 2c). Pathological response (HR 7.144, 95% CI 1.358–131.592, p = 0.016) was significantly correlated with PRS in univariate analysis. Multivariate analysis revealed that tumor size (HR 3.108 × 10^9, 95% CI 1.662–7.5 × 10^179, p = 0.024), lymph node (HR 1.772 × 10^10, 95% CI 1.806–unparsable, p = 0.028), pathological response (HR 300.204, 95% CI 7.824–52,372.311, p < 0.001), and metastatic site (HR 15.037, 95% CI 2.182–159.623, p = 0.005) were independent prognostic factors for survival (Table 4). Among the 20 patients with TNBC, there was no significant difference in PRS in relation to pathological response (p = 0.329, log-rank) (Fig. 2d). In univariate analysis, only

| Table 2 Univariate and multivariate analyses with respect to relapse-free survival in breast cancer subtypes |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Univariate analysis | Multivariate analysis | Hazard ratio | 95% CI | p-value | Hazard ratio | 95% CI | p-value |
|---------------------|----------------------|--------------|--------|---------|--------------|--------|---------|
| All breast cancer (n = 237) | | | | | | | |
| Age (≤ 56) | 1.491 | 0.877–2.565 | 0.141 | 2.130 | 0.828–4.847 | 0.111 |
| Menopause (−) | 1.220 | 0.709–2.074 | 0.467 | 0.703 | 0.310–1.802 | 0.439 |
| Tumor size (> 2) | 1.679 | 0.738–4.830 | 0.236 | 1.509 | 0.640–4.436 | 0.372 |
| Lymph node (+) | 1.720 | 0.904–3.620 | 0.102 | 1.829 | 0.950–3.880 | 0.072 |
| Nuclear grade (3) | 0.899 | 0.427–1.713 | 0.758 | 1.452 | 0.642–3.064 | 0.356 |
| Ki67 (> 14) | 0.553 | 0.325–0.945 | 0.031 | 0.548 | 0.300–0.991 | 0.047 |
| Pathological response (non-pCR) | 2.046 | 1.143–3.891 | 0.015 | 1.886 | 1.005–3.746 | 0.048 |
| Luminal (n = 93) | | | | | | | |
| Age (≤ 56) | 1.223 | 0.537–2.871 | 0.631 | 5.007 | 0.988–20.715 | 0.052 |
| Menopause (−) | 0.864 | 0.367–1.974 | 0.730 | 0.268 | 0.072–1.290 | 0.094 |
| Tumor size (> 2) | 2.077 | 0.607–13.00 | 0.276 | 2.444 | 0.650–16.093 | 0.206 |
| Lymph node (+) | 3.188 | 0.935–19.94 | 0.066 | 4.842 | 1.336–31.230 | 0.013 |
| Nuclear grade (3) | 1.110 | 0.365–2.799 | 0.839 | 2.386 | 0.640–8.278 | 0.187 |
| Ki67 (> 14) | 0.587 | 0.256–1.360 | 0.209 | 0.336 | 0.119–0.906 | 0.031 |
| Pathological response (non-pCR) | 1.178 | 0.487–3.281 | 0.728 | 1.403 | 0.513–4.387 | 0.523 |
| HER2-enriched (n = 43) | | | | | | | |
| Age (≤ 56) | 2.124 | 0.559–8.614 | 0.262 | 1.059 | 0.050–9.029 | 0.962 |
| Menopause (−) | 1.877 | 0.463–7.126 | 0.359 | 6.241 | 0.451–175.683 | 0.177 |
| Tumor size (> 2) | 1.063 | 0.195–19.750 | 0.953 | 0.281 | 0.024–6.713 | 0.368 |
| Lymph node (+) | 1.859 | 0.447–12.523 | 0.417 | 1.683 | 0.292–15.232 | 0.579 |
| Nuclear grade (3) | 0.405 | 0.022–2.245 | 0.344 | 0.163 | 0.007–1.387 | 0.103 |
| Ki67 (> 14) | 0.607 | 0.160–2.460 | 0.464 | 0.868 | 0.140–6.349 | 0.882 |
| Pathological response (non-pCR) | 1.156 | 0.412–6.289 | 0.508 | 4.430 | 0.569–55.264 | 0.163 |
| TNBC (n = 80) | | | | | | | |
| Age (≤ 56) | 1.483 | 0.611–3.697 | 0.381 | 2.697 | 0.542–10.890 | 0.205 |
| Menopause (−) | 1.318 | 0.515–3.195 | 0.550 | 0.539 | 0.136–2.660 | 0.416 |
| Tumor size (> 2) | 1.155 | 0.333–7.263 | 0.844 | 0.643 | 0.160–4.296 | 0.596 |
| Lymph node (+) | 1.065 | 0.411–3.282 | 0.904 | 0.625 | 0.214–2.083 | 0.421 |
| Nuclear grade (3) | 1.098 | 0.314–2.999 | 0.868 | 2.909 | 0.679–11.523 | 0.143 |
| Ki67 (> 14) | 0.488 | 0.202–1.250 | 0.130 | 0.486 | 0.150–1.518 | 0.213 |
| Pathological response (non-pCR) | 4.251 | 1.557–14.857 | 0.004 | 5.013 | 1.612–19.386 | 0.004 |

Values in parentheses are 95% confidence intervals.

CI, confidence interval; pCR, pathological complete response; TNBC, triple-negative breast cancer.
Ki-67 (HR 4.242, 95% CI 1.078–28.056, p = 0.038) was significantly correlated with PRS. Multivariate analysis revealed that Ki-67 (HR 51.171, 95% CI 1.769–4346.194, p = 0.020) and metastatic site (HR 13.318, 95% CI 1.021–540.473, p = 0.048) were independent prognostic factors for survival. Because the number of patients with luminal-HER2 (n = 3) and HER2-enriched (n = 9) breast cancer was small, statistical analyses were not performed (Table 4).

**Discussion**

Classification of breast cancer intrinsic subtypes is useful in the prediction of therapeutic response and prognosis, mainly for the primary tumor. However, there have been few studies that stratify patients with recurrent breast cancer by intrinsic subtype [11–14]. These studies reported that ER-negative breast cancer or TNBC was significantly associated with poor prognosis. However, these investigations were not examined by each intrinsic subtype of breast cancer. In our study, among 20 TNBC patients with recurrence, low Ki-67 and metastatic site (local or bone) were significantly associated with good prognosis. In addition, although these studies included patients who were not treated with NAC, NAC is the gold standard of care for breast cancer. Our study is the first to investigate the prognostic factor of recurrent breast cancer after treatment with NAC and surgery in all intrinsic subtypes.

In the present study, patients with HER2-enriched breast cancer and TNBC had significantly higher pCR rates among all the patient groups. In particular, RFS after NAC and surgery was significantly longer for patients with TNBC who achieved pCR. Because some
previous studies suggested that the treatment response of highly malignant breast cancers, namely, HER2-enriched breast cancer and TNBC, is related to the number of tumor-infiltrating lymphocytes, both are considered to have high immunoactivity [24–27]. However, among patients who relapsed after surgery, no association between each subtype and clinicopathological features was found, including pathological response. Additionally, PRS was significantly better for patients with luminal breast cancer who achieved pCR, but not for those with TNBC. Luminal breast cancer comprises epithelial cells and has low invasion capability, whereas TNBC comprises mesenchymal cells, and has high invasion and migration capabilities [28, 29]. Compared to that of the primary tumor, relapsed breast cancer after NAC and surgery often acquires resistance to chemotherapy; thus, the prognosis may depend on the degree of invasion or migration rather than immunoactivity.

In terms of metastatic sites at relapse, distant metastasis, such as the liver or lung, excluding bone, is considered life-threatening and is often treated with chemotherapy based on the first-line regimen [30]. Even in our study, the metastatic site was an independent prognostic factor for both the luminal and TNBC subtypes. A recent study reported that disease-free interval was significantly associated with PRS, particularly in luminal breast cancer [31]. However, in this study, there was no relationship between RFS and PRS for both luminal breast cancer and TNBC. The reason for this inconsistent result may be that we only analyzed patients treated with NAC. Future studies may find that cyclin-dependent kinase 4/6 inhibitors (palbociclib, ribociclib, and abemaciclib) could be an effective treatment modality for cases of recurrent luminal cancer that did not achieve pCR.

In the present study, patients were classified into subtypes according to findings from core needle biopsy.
before NAC, while it has been reported that the receptor status of the relapsed breast cancer after NAC may change [32, 33]. Re-biopsy after recurrence is recommended because it may improve information for creating an individualized treatment plan [16]. However, re-biopsy of distant recurrence is often difficult, including the brain or bone, and we did not analyze it in this study. Instead of core needle biopsy of the tumor, liquid biopsy, such as of circulating tumor cells, cell-free DNA, and tumor-derived exosomes, is likely to influence the treatment strategy in the future [34, 35].

A potential limitation of the current study is that we did not evaluate luminal-HER2, and HER2-enriched breast cancer in detail because of the small sample size of our study. Further prospective cohort studies are therefore needed to address these limitations.

### Conclusions

This study is the first to investigate the PRS of patients treated with NAC and subsequent curative surgery in each intrinsic molecular subtype. It is important to separately evaluate the prognostic factors for each intrinsic subtype in patients with recurrent breast cancer following NAC and surgery.

### Abbreviations

NAC: neoadjuvant chemotherapy; pCR: pathological complete response; PRS: post-recurrence survival; HER: human epidermal growth factor receptor; TNBC: triple-negative breast cancer

### Table 4 Univariate and multivariate analyses with respect to post-recurrence survival in breast cancer subtypes

| hazard ratio | 95% CI   | p-value | hazard ratio | 95% CI   | p-value |
|--------------|---------|---------|--------------|---------|---------|
| All breast cancer (n = 55) |          |         |              |         |         |
| Age (≤ 56) | 0.639 | 0.301–3.313 | 0.231 | 0.369 | 0.076–1.337 | 0.135 |
| Menopause (−) | 0.651 | 0.289–1.380 | 0.268 | 1.936 | 0.518–9.476 | 0.340 |
| Tumor size (> 2) | 2.262 | 0.666–14.138 | 0.216 | 5.534 | 1.251–39.387 | 0.023 |
| Lymph node (+) | 0.760 | 0.288–2.611 | 0.627 | 0.295 | 0.083–1.207 | 0.086 |
| Nuclear grade (3) | 1.308 | 0.516–2.921 | 0.547 | 0.751 | 0.256–2.016 | 0.577 |
| Ki67 (> 14) | 1.797 | 0.859–3.881 | 0.120 | 2.606 | 1.028–6.780 | 0.044 |
| Pathological response (non-pCR) | 3.321 | 1.264–11.437 | 0.013 | 4.355 | 1.438–16.871 | 0.008 |
| Metastasis site (distant) | 1.415 | 0.666–2.950 | 0.049 | 2.496 | 1.007–6.126 | 0.048 |
| Relapse-free survival (< 2 years) | 2.439 | 1.005–7.269 | 0.049 | 1.938 | 0.681–6.598 | 0.225 |
| Luminal (n = 23) |          |         |              |         |         |
| Age (≤ 56) | 1.228 | 0.382–3.948 | 0.724 | 5.415 | 0.194–115.713 | 0.275 |
| Menopause (−) | 1.252 | 0.369–3.941 | 0.704 | 1.614 | 0.104–46.371 | 0.736 |
| Tumor size (> 2) | 6.235 × 107 | 0.822–0.822 | 0.073 | 3.108 × 109 | 1.662–7.5 × 10179 | 0.024 |
| Lymph node (+) | 5.735 × 107 | 0.561–0.561 | 0.135 | 1.772 × 1010 | 1.806-unparsable | 0.028 |
| Nuclear grade (3) | 1.198 | 0.265–4.042 | 0.791 | 0.436 | 0.034–4.914 | 0.477 |
| Ki67 (> 14) | 1.118 | 0.345–3.648 | 0.849 | 0.982 | 0.180–4.386 | 0.982 |
| Pathological response (non-pCR) | 7.144 | 1.358–131.592 | 0.016 | 300.204 | 7.824–52,372,311 | < 0.001 |
| Metastatic site (distant) | 2.520 | 0.740–7.971 | 0.132 | 15.037 | 2.182–159.623 | 0.005 |
| Relapse-free survival (< 2 years) | 2.175 | 0.647–9.825 | 0.219 | 0.165 | 0.012–2.177 | 0.160 |
| TNBC (n = 20) |          |         |              |         |         |
| Age (≤ 56) | 0.936 | 0.291–3.018 | 0.910 | 0.578 | 0.011–20.101 | 0.759 |
| Menopause (−) | 1.025 | 0.299–3.258 | 0.967 | 0.919 | 0.022–36.398 | 0.962 |
| Tumor size (> 2) | 0.948 | 0.222–6.663 | 0.949 | 2.141 | 0.100–93.753 | 0.639 |
| Lymph node (+) | 0.507 | 0.133–2.415 | 0.360 | 1.083 | 0.049–53.468 | 0.963 |
| Nuclear grade (3) | 1.841 | 0.479–6.155 | 0.350 | 0.109 | 0.003–1.421 | 0.094 |
| Ki67 (> 14) | 4.242 | 1.078–28.056 | 0.038 | 51.171 | 1.769–4346.194 | 0.020 |
| Pathological response (non-pCR) | 2.110 | 0.547–13.871 | 0.303 | 1.813 | 0.045–56.645 | 0.733 |
| Metastatic site (distant) | 1.569 | 0.486–5.070 | 0.442 | 13.318 | 1.021–540.473 | 0.048 |
| Relapse-free survival (< 2 years) | 7.070 × 107 | 0.812–8.812 | 0.074 | 2.294 × 107 | 0.027–3.0 × 10289 | 0.454 |

Values in parentheses are 95% confidence intervals.

CI: confidence interval, pCR: pathological complete response, TNBC: triple-negative breast cancer.
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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The datasets supporting the conclusions of this article is included within the article.

Consent for publication
Written informed consent was obtained from all patients.

Ethics approval and consent to participate
Written informed consent was obtained from all subjects. This research conformed to the provisions of the Declaration of Helsinki in 2013. All patients were informed of the investigational nature of this study and provided their written, informed consent. The study protocol was approved by the Ethics Committee of Osaka City University (#926).

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