





## ORIGINAL ARTICLE

# Pathologic method for extracting good prognosis group in triple-negative breast cancer after neoadjuvant chemotherapy

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## Abstract

The area of residual tumor (ART) is a prognostic factor in patients treated with neoadjuvant chemotherapy (NAC) for lung, pancreatic, and rectal cancers. This study aimed to evaluate the usefulness of ART as a method for predicting the prognosis of triple-negative breast cancer (TNBC) patients after NAC. We included 143 patients with TNBC treated with NAC. The ART at the maximum cut surface of the residual tumor was measured. We divided the patients into three groups: ART-0 (ART = 0 mm<sup>2</sup>), ART-low (0 mm<sup>2</sup> < ART ≤ 136mm<sup>2</sup>), and ART-high (ART > 136 mm<sup>2</sup>), and compared their clinicopathologic factors and prognosis. There were no significant differences in either recurrence-free survival (RFS) or overall survival (OS) between ART-0 and ART-low; however, the ART-high group had significantly shorter RFS and OS than the ART-0 and ART-low groups. Multivariate analysis showed that ART-0 and -low and ypN(-) were independent favorable prognostic factors for RFS. Groups with both ART-low and ypN(-) as well as those with ART-0 and ypN(-) showed significantly longer OS and RFS than the other groups ( $P < .05$ ). Moreover, there was no significant difference in the RFS and OS between the ART-0 and ypN(-) groups and the ART-low and ypN(-) groups ( $P = .249$  and  $P = .554$ , respectively). We concluded that ART is a candidate histopathological evaluation method for predicting the prognosis of TNBC patients treated with NAC. Furthermore, postoperative chemotherapy could be omitted in patients with ART-0 and ypN(-) (pathological complete response) and those with ART-low and ypN(-).

## KEYWORDS

breast neoplasm, evaluation criteria in solid tumors, neoadjuvant therapy, pathology

**Abbreviations:** ART, area of residual tumor; CI, confidence interval; H&E, hematoxylin and eosin; HER2, hormone receptor-positive human epidermal growth factor receptor 2; JBCS, Japanese Breast Cancer Society; MPS, Miller and Payne system; NAC, neoadjuvant chemotherapy; OS, overall survival; pCR, pathological complete response; RFS, recurrence-free survival; ROC, receiver operating characteristic; TNBC, triple-negative breast cancer.

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## 1 | INTRODUCTION

In breast cancer, NAC is often chosen because it allows minimally invasive surgery due to tumor shrinkage and allows postoperative response-guided therapy depending on whether a pCR has been achieved.

In contrast to the HER2<sup>-</sup> type, patients with TNBC tend to be treated with NAC more aggressively because of the higher likelihood of achieving pCR with NAC and the better prognostic effect of capecitabine as response-guided therapy.<sup>1,2</sup>

It is important to accurately estimate the effect of NAC to predict prognosis and determine postoperative treatment strategies. Histopathological methods for determining the therapeutic effect of NAC are broadly classified into estimation of the tumor bed based on histological findings and evaluation by the area ratio to the residual cancer, and absolute evaluation of the amount of residual cancer.

The former method includes the MPS, Sataloff classification, the National Surgical Adjuvant Breast and Bowel Project B18 trial (NSABP-B18), and the criteria for determining the histological response to treatment proposed by the JBCS.<sup>3-7</sup> All of these classify the responses between a complete response and no response into several categories. Another prediction model that combines it with other pathological factors is the residual cancer burden and residual disease in the breast and nodes.<sup>8,9</sup> However, it is sometimes difficult to accurately estimate the tumor bed; the latter methods include ypTNM classification, clinicopathologic stage + estrogen receptor status, and grade staging, and ART. In every classification, it has been reported that the prognosis can be stratified according to the degree of treatment response.<sup>10-13</sup>

As it was found that pCR has a more favorable prognosis than non-pCR, and that additional treatment in non-pCR groups can improve the prognosis, pCR/non-pCR is an important indicator of breast cancer.<sup>14,15</sup> However, there is a large range in the amount of cancer remaining in the non-pCR groups, and it is possible that there is a population within that group that can be omitted from additional treatment.

Our institution has reported measurement of ART as a new objective and quantitative pathological evaluation method for assessing residual tumor in post-NAC resections of lung, rectal, and pancreatic cancers.<sup>16-18</sup> The measurement of ART is an absolute evaluation of the residual tumor area of a specimen after NAC by calculating it on a digital image, which has the potential to solve the above problems. Therefore, ART could provide more objective and evidence-based pathological information of tumors that receive preoperative therapy.

The purpose of this study was to evaluate the usefulness of ART as a method for predicting the prognosis of TNBC and to identify populations other than pCR that can be excluded from postoperative chemotherapy.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

Patients with TNBC without distant metastasis who underwent surgery at the National Cancer Center Hospital East and who received NAC between January 2008 and December 2017 were included in the study. Patients without intramammary lesions and those with postoperative changes to the non-TNBC subtype were excluded (Figure S1).

We collected clinicopathological information (age, sex, menstrual status, imaging findings, preoperative treatment details, surgical findings [surgical procedure, etc.], pathological findings [histological type and stage, etc.], tumor size, histological grade, course of treatment, prognosis, recurrence, and death) of the patients from their medical records. This study used samples for which the National Cancer Center provided comprehensive consent and was carried out in compliance with relevant guidelines and regulations. The Ethical Review Committee of the National Cancer Center approved the study protocol. (No. 2021-021).

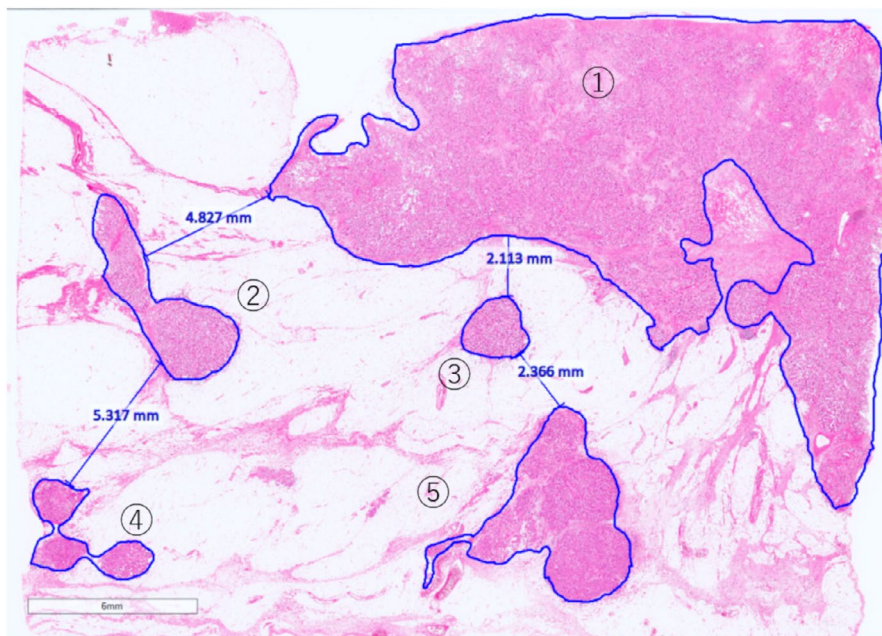
### 2.2 | Method of ART measurement

We made whole area histological sections of partial mastectomy specimens by dividing them into 5-10-mm sections. We sliced the whole mastectomy specimen into 5-10-mm sections and made slides of sections of the area where we thought the mass was, the area around the mass, and the area between the mass and the nipple. We observed all H&E slides of the largest slice of the residual tumor under a microscope and outlined them for landmarks with a marker pen to identify residual tumor cells. Degenerated tumor cells with nuclei and cytoplasm were defined as residual tumor cells; necrotic tumor cells and intraductal components were excluded. If a group of residual tumor cells was 2 mm away from the neighboring group, it was considered a separate residual tumor tracing the contour of the tumor nest. The total area of the largest slice was defined as ART. The first breast surgeon (Y.E) measured ART in each group based on the pathology report, and then one pathologist (T.N) specializing in breast cancer confirmed it. Figure 1 shows the actual measurement method: the five lesions on this slide were more than 2 mm apart, and the area of each was measured and summed. The total area of this slide was 225.54 mm<sup>2</sup>. The same measurement was made for all slides of the maximum split surface, and the total value was ART for each group.

### 2.3 | Immunohistochemistry

Tissue sections measuring 4 μm thick from each formalin-fixed paraffin-embedded block were cut on separate Starfrost slides

**FIGURE 1** Measurement of area of residual tumor using partial mastectomy specimens. This slide had five lesions more than 2 mm apart, with a total area of 225.54 mm<sup>2</sup>



①181.074mm<sup>2</sup>

②12.310mm<sup>2</sup>

③3.781mm<sup>2</sup>

④6.077mm<sup>2</sup>

⑤22.295 mm<sup>2</sup>

total :225.54mm<sup>2</sup>

(5126Y; Muto Chemical). Using the Bench-Mark XT instrument (Roche Tissue Diagnostics) and a preprogrammed protocol, each slide was stained with anti-estrogen receptor (SP1; Roche Tissue Diagnostics), anti-progesterone receptor (1E2; Roche Tissue Diagnostics), and anti-HER2 (4B5; progesterone receptor) rabbit mAbs. Slides for Ki-67 were stained with Ki-67 mouse mAbs (MIB-1; Agilent) using Autostainer Link48 (Wakenyaku Co. Ltd).

## 2.4 | Statistics analysis

We used RFS and OS to evaluate the effectiveness of ART as independent prognostic factors. Recurrence-free survival was defined as the time from surgery to recurrence or death from any cause. Overall survival was defined as the time from surgery to death due to any cause. Both RFS and OS were estimated using the Kaplan-Meier method and log-rank test, and univariate and multivariate Cox regression models were used to compare the groups. To dichotomize the ART (low or high), the ROC curve was estimated using Cox regression for RFS, and the cut-off value was determined based on the lower distances to the top-left corner of the ROC curve. To compare clinicopathological characteristics, Fisher's exact test was applied for categorical variables, and the *t* test for continuous variables.

Statistical analyses were undertaken using JMP version 15 (SAS Institute) or EZR (Saitama Medical Center, Jichi Medical University), which is a modified version of R Commander (The R Foundation for Statistical Computing) designed to add statistical functions frequently used in biostatistics.<sup>19</sup> We defined the statistical significance of two-sided *P*-value of .05.

## 3 | RESULTS

### 3.1 | Clinicopathological features

Table 1 shows the clinicopathological characteristics of the patients who received NAC. All patients were women, and their median age was 57 years (range, 28–82 years); 136 (95.1%) patients were in clinical stage 2 or higher. Neoadjuvant chemotherapy consisted of an anthracycline plus taxane regimen in 115 patients (80.4%) and other chemotherapy regimens in 28 patients (19.6%). No patients receiving postoperative capecitabine were included in the study. Pathological T stages were 0, 1, 2, 3, and 4 in 7 (16.2%), 3 (7.0%), and 4 (18.6%) patients, respectively. In surgical specimens, lymph node metastasis was confirmed in 39 (27.3%) patients. Tumor downstaging was observed in 127 of the 143 patients (88.8%). For the type of breast cancer present, 130 were ductal carcinoma, five were apocrine

**TABLE 1** Characteristics of 143 patients with triple-negative breast cancer

| Characteristics          | Total N = 143 |
|--------------------------|---------------|
| Age (y)                  | 57 (28-82)    |
| Menopausal status        |               |
| Pre                      | 67 (46.9)     |
| Post                     | 75 (52.5)     |
| Unknown                  | 1 (0.7)       |
| cT                       |               |
| 1/2/3/4                  | 11/93/27/12   |
| cN                       |               |
| Negative                 | 50 (35.0)     |
| Positive                 | 93 (65.0)     |
| cStage                   |               |
| 1/2/3                    | 7/88/48       |
| Operation                |               |
| BCS                      | 66 (46.2)     |
| Bt                       | 77 (53.8)     |
| SN                       | 42 (29.4)     |
| Ax                       | 101 (70.6)    |
| Chemotherapy             |               |
| Anthracycline and taxane | 115(80.4)     |
| Other                    | 28 (19.6)     |
| Radiotherapy             |               |
| Yes                      | 112 (78.3)    |
| No                       | 31 (21.7)     |
| Lymphatic permeation     |               |
| Absent                   | 131 (91.6)    |
| Present                  | 12 (8.4)      |
| Vascular invasion        |               |
| Absent                   | 131 (91.6)    |
| Present                  | 12 (8.4)      |
| MPS                      |               |
| 1/2/3/4/5                | 16/47/23/8/49 |
| JBCS grading system      |               |
| 0/1/2/3                  | 16/68/10/49   |

Note: Data are shown as number (%) among the number of patients in each group or median (range).

Abbreviations: Ax, axillary lymph node dissection; BCS, breast-conserving surgery; Bt, breast mastectomy; JBCS, Japanese Breast Cancer Society; MPS, Miller Payne system; SN, sentinel lymph node biopsy.

carcinoma, six were metaplastic carcinoma, and two were lobular carcinoma.

### 3.2 | Measurement of ART

Representative H&E slides are shown in Figure 2(A-D). Figure 2(A) shows a slide of a portion of the largest slice of a case in which many

cancer cells remained after NAC and Figure 2(B) is a larger image of Figure 2(A). The ART in this case was 333.8 mm<sup>2</sup>. No fibrosis or necrosis was observed. Figure 2(C) shows a case where only a small number of cancer cells remain; Figure 2(D) is a larger image of Figure 2(C). The ART was 2.5 mm<sup>2</sup>. A high degree of fibrosis was observed between the foci. In areas where treatment was effective and tumor cells disappeared, histiocytes containing hemosiderin, cholesterol clefts, and foam cell infiltration were also found in these areas (Figure 2E, F). There were 49 patients with ART-0, and the median ART was 29.1 mm<sup>2</sup> (range, 0.0-1830.1 mm<sup>2</sup>) (Figure 2G).

We determined the cut-off value to separate ART-low and ART-high as 136 mm<sup>2</sup> using the ROC curve for RFS, excluding the 49 patients with ART-0 (Figure 3).

### 3.3 | Relationship between ART and Ki-67 index

We evaluated the relationship between ART and Ki-67 index before and after NAC. Ki-67 index before NAC was significantly higher in the ART-0 and ART-high groups than in the ART-low group (Figure 4A;  $P = .012$  and  $P = .031$ , respectively). Ki-67 index after NAC was also significantly higher in the ART-high group than in the ART-low group. (Figure 4B;  $P = .004$ ). The changes in Ki-67 index before and after chemotherapy were compared between the ART-low and ART-high groups. In both groups, the Ki-67 index significantly decreased after chemotherapy (Figure 4C,D).

### 3.4 | Differences in clinicopathological characteristics among ART status

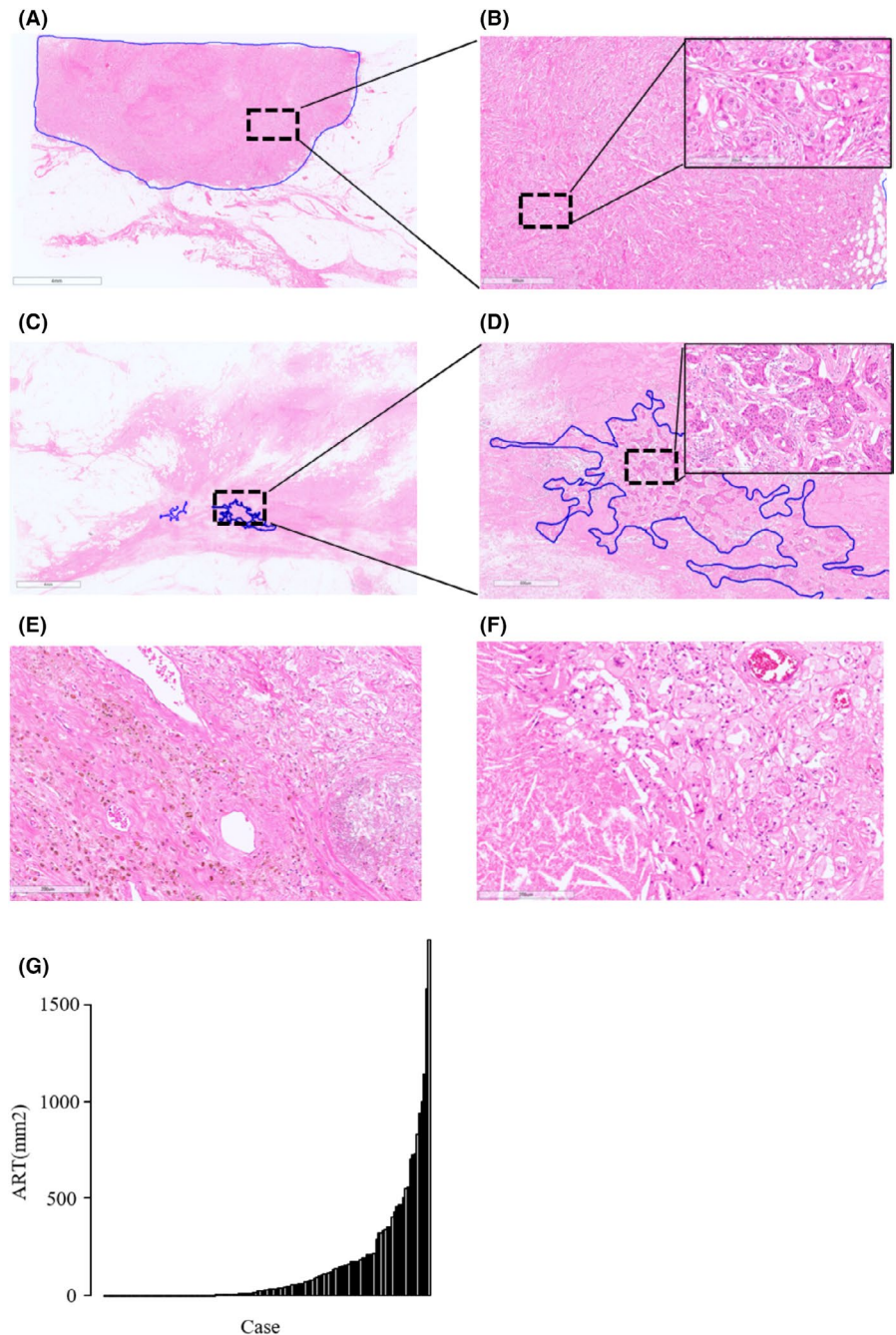
The relationship between ART status and clinicopathological characteristics of the patients is shown in Table 2. There were no significant differences in clinicopathological factors between ART-0 and ART-low except for cT. However, groups with ART-high contained more lymphatic permeation, vascular invasion, and residual lymph node metastases than the ART low and ART high groups (all  $P < .05$ ).

### 3.5 | Relapse-free survival and OS analysis

The median observation period was 68.6 months. The 5-year RFS for ART-0, ART-low, and ART-high were 87.4% (95% CI, 74.1%-94.1%), 88.2% (95% CI, 75.5%-94.5%), and 50.7% (95% CI, 34.5%-64.8%), respectively. The 5-year OS for ART-0, ART-low, and ART-high were 87.3% (95% CI, 73.8%-94.1%), 94.3% (95% CI, 83.5%-98.1%), and 56.3% (95% CI, 38.4%-70.8%), respectively. There was no difference in RFS and OS between the ART-0 and ART-low groups, and the ART-high group had a significantly shorter OS and RFS than both groups (Figures 5A,B and S2).

In univariate analysis, ART, cT, ypN, ly, and v factors were significant prognostic factors. Multivariate analysis showed that ART-0 + ART-low and ypN(-) were independent favorable prognostic

**FIGURE 2** Representative images of breast cancer cells. (A) A case of abundant cancer cells remaining after neoadjuvant chemotherapy (NAC). (B) Higher magnification image of square in (A). (C) A case with only a small number of cancer cells remaining after NAC. (D) Higher magnification image square in (C). (E) Histopathologic features of breast cancer treated by NAC: tearing and degeneration of collagen fibers, histiocytes containing hemosiderin. (F) Histopathologic features of breast cancer treated by NAC: cholesterol clefts and foamy histiocytes containing hemosiderin. (G) Histogram of area of residual tumor (ART) for each case. The X and Y axes represent patient number and ART, respectively



factors for RFS, and that the prognosis was similar between ART-0 and ART-low (Table 3).

When examined within ypT1, ART-low has a better prognosis than ART-high with a significant difference, and within ypT2, ART-low tends to have a better prognosis than ART-high, but not significantly (Figure S3A,B). When we examined the ypN(-) group, there was no statistically significant difference between ART-low and ART-high, but among the ypN+ group, ART-low has a statistically superior prognosis to ART-high (Figure S3C,D).

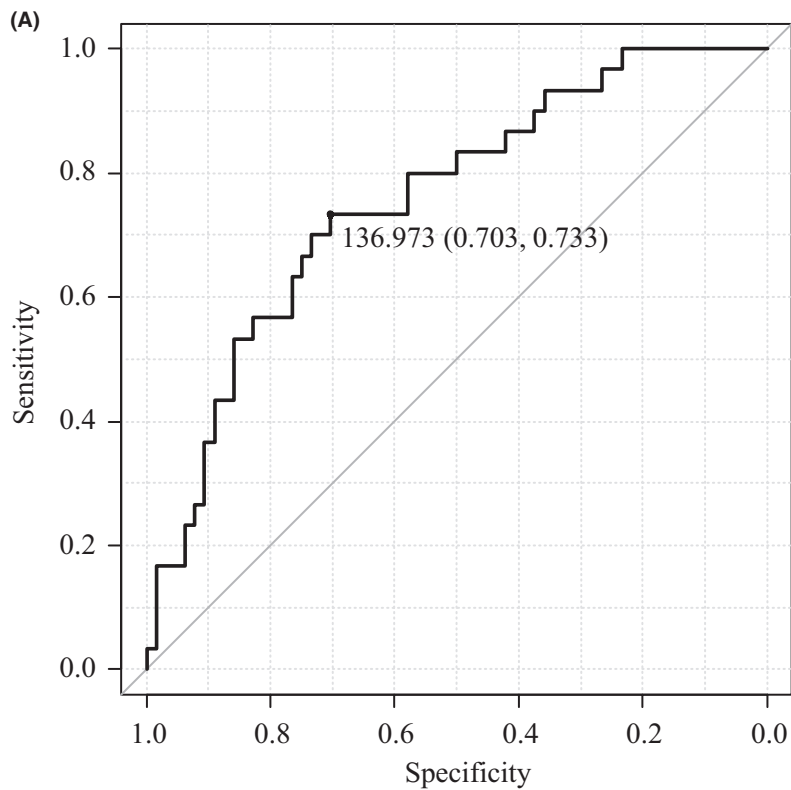
### 3.6 | Comparison with other assessment systems

In order to compare ART with other assessment systems (MPS, ypT classification, and JBCS grading system), we undertook a univariate

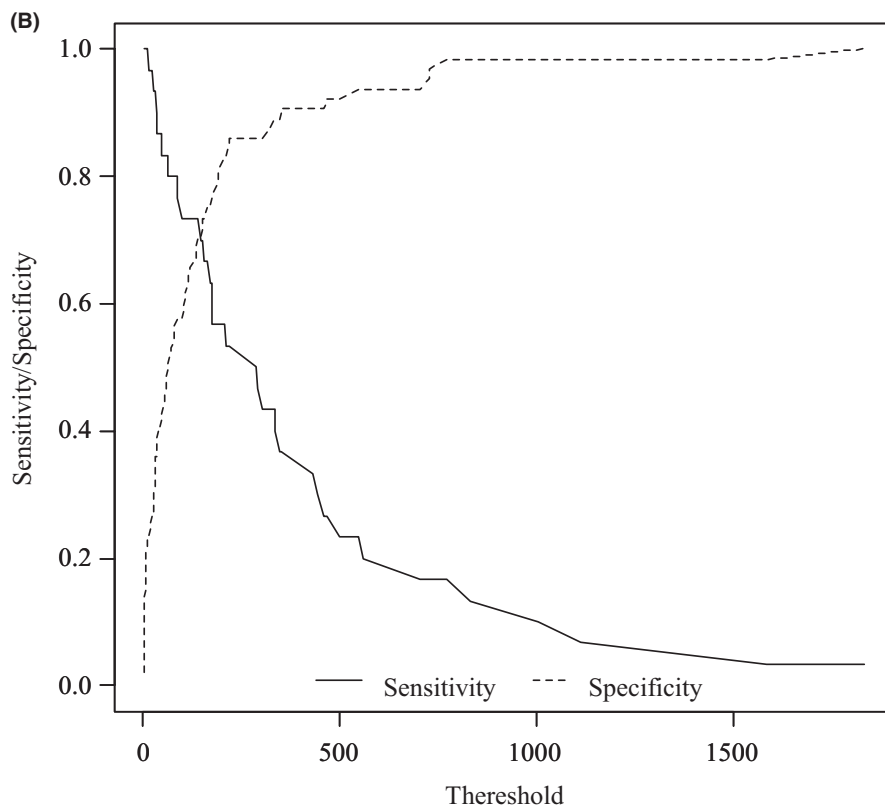
Cox regression analysis and the same multivariate analysis as in Table 3 for each evaluation system (cT, ypN, ly, v, multivariate analysis for each evaluation system). The MPS, ypT classification, and JBCS grading system were reclassified into two groups. All grading systems were statistically significant in the univariate analysis. T ART and ypT remained when multivariate analysis was carried out, and ART was more predictive of prognosis than ypT (Table 4).

### 3.7 | Assessment of the combination of ART and ypN

We combined ART and ypN factors and divided them into three groups: ARTN- $\alpha$  (ART-0 and ypN[-]), ARTN- $\beta$  (ART  $\leq$  136 and ypN[-]), and ARTN- $\gamma$  (ART > 136 or ypN[+]). There was no significant



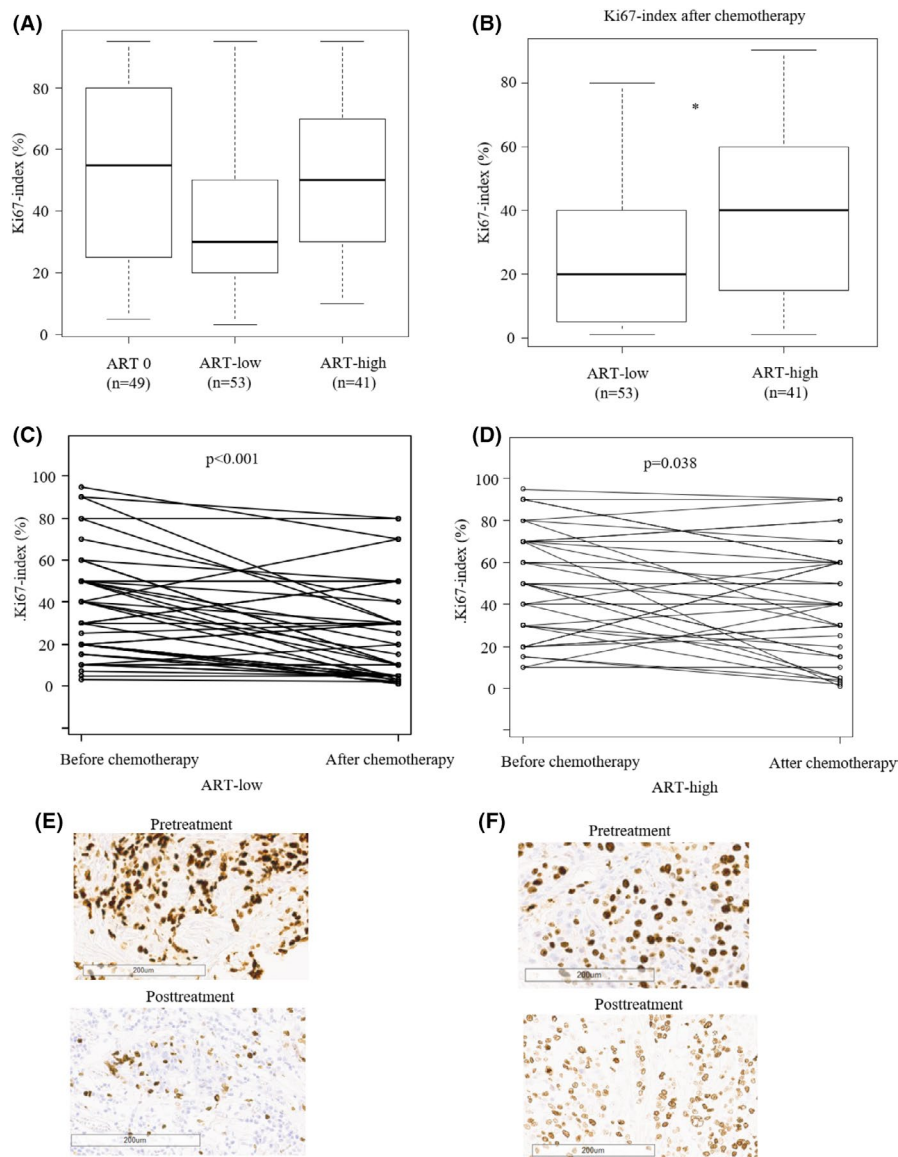
**FIGURE 3** Determination of the area of residual tumor threshold in triple-negative breast cancer. (A) Receiver operating characteristic curve. (B) Sensitivity/specificity per threshold



difference in RFS or OS between ARTN- $\alpha$  and ARTN- $\beta$ , and ARTN2- $\gamma$  had significantly worse RFS and OS compared to both groups (Figure 5C,D).

As there was no difference between the ART-0/ypN(-) and ART-low/ypN(-) groups, we combined these groups together and divided them into ARTN-I (ART  $\leq$  136 and ypN[-]), ARTN-II (ART  $\leq$  136/

**FIGURE 4** Relationship between area of residual tumor (ART) and Ki-67 index in triple-negative breast cancer specimens. (A) Ki-67 index before neoadjuvant chemotherapy (NAC) in ART-0, ART-low, and ART-high groups. \* $P = .012$ , \*\* $P = .031$ . (B) Ki-67 index after NAC in ART-low and ART-high groups.  $P = .004$ . (C) Change in Ki-67 index before and after NAC in ART-low group. (D) Change in Ki-67 index before and after NAC in ART-high group. (E) A case with a significant decrease in Ki-67 index before and after NAC. (F) A case with a little change in Ki-67 index before and after NAC



ypN[+] or ART > 136/ypN[-]), and ARTN-III (ART > 136 and ypN[+]), which clearly separated the prognoses of the three groups (Figure 5E,F).

## 4 | DISCUSSION

In this study, we found that the measurement of ART could stratify the prognosis of TNBC after NAC. Multivariate analysis revealed that ART-0 and ART-low were independent prognostic factors for RFS. In addition, we compared ART with other pathological evaluation methods: ypT classification, JBCS grading system, and MPS. As a result, ART was useful for extracting groups with favorable prognoses (Table 4). The ART method does not require the estimation of the tumor bed or the area ratio between the tumor bed and the residual cancer cell area, and the use of digital pathology images facilitates the measurement of the residual cancer cell area, making the method objective, quantitative, and easily agreed upon by pathologists.

In breast cancer, NAC is especially important for TNBC because of the greater improvement in prognosis by capecitabine in non-pCR TNBC than in hormone receptor-positive breast cancer.<sup>2</sup> Capecitabine is currently omitted in patients with pCR (ypT0/is, ypN[-]), and our study suggests that capecitabine can be omitted not only in pCR patients but also in ART-low, ypN(-) patients with TNBC. As capecitabine is given for approximately 6 months and causes grades 1–3 hand-foot syndrome in 70% of patients, reducing their quality of life, it would be beneficial to explore populations in which capecitabine could be omitted in the non-pCR group.<sup>2</sup>

Some researchers have reported on the correlation between therapeutic effects and pretreatment Ki-67 index. Fasching et al. reported that the pretreatment Ki-67 index was an independent predictor of pCR in types other than TNBC.<sup>20</sup> On the contrary, other studies have reported that pretreatment Ki-67 index was not a significant predictor of pCR.<sup>21,22</sup> In our study, the pretreatment Ki-67 index could not be a predictor of treatment effect. This could be due to the fact that both the ART-0 and ART-high groups had high Ki-67

TABLE 2 Comparison of clinicopathological features of 143 women with triple-negative breast cancer, according to area of residual tumor (ART) status

| Characteristic                  | ART status     |                        |                    | P value <sup>a</sup> |        |
|---------------------------------|----------------|------------------------|--------------------|----------------------|--------|
|                                 | ART-0 (n = 49) | 0 < ART ≤ 136 (n = 53) | ART > 136 (n = 41) |                      |        |
| Age (y)                         | 58 (28–77)     | 56 (30–80)             | 58 (31–82)         | .954                 | .7940  |
| Menopausal state                |                |                        |                    |                      |        |
| Pre                             | 21 (42.9)      | 24 (45.3)              | 22 (55.0)          | .844                 | .4060  |
| Post                            | 28 (57.1)      | 29 (54.7)              | 18 (45.0)          |                      |        |
| cT                              |                |                        |                    |                      |        |
| T1/2                            | 43 (87.8)      | 36 (67.9)              | 25 (61.0)          | .019                 | .5190  |
| T3/4                            | 6 (12.2)       | 17 (32.1)              | 16 (39.0)          |                      |        |
| cN                              |                |                        |                    |                      |        |
| N–                              | 19 (38.8)      | 21 (39.6)              | 10 (24.4)          | 1.000                | .1290  |
| N+                              | 30 (24.4)      | 32 (60.4)              | 31 (75.6)          |                      |        |
| Pretreatment Ki-67              |                |                        |                    |                      |        |
| ≤15                             | 5 (10.2)       | 10 (18.9)              | 4 (9.8)            | .261                 | .3160  |
| >15                             | 37 (75.5)      | 35 (66.0)              | 33 (80.5)          |                      |        |
| Unknown                         | 7(14.3)        | 8 (15.1)               | 4 (9.8)            |                      |        |
| Pretreatment histological grade |                |                        |                    |                      |        |
| 1                               | 2 (4.1)        | 2 (3.8)                | 1 (2.4)            | 1.000                | 1.0000 |
| 2                               | 17 (34.7)      | 24 (45.3)              | 20 (48.8)          |                      |        |
| 3                               | 9 (18.4)       | 11 (20.8)              | 8 (19.5)           |                      |        |
| Unknown                         | 21(42.9)       | 16(30.2)               | 12 (29.3)          |                      |        |
| ypN                             |                |                        |                    |                      |        |
| N–                              | 44 (89.8)      | 40 (75.5)              | 20 (48.8)          | .0715                | .0097  |
| N+                              | 5 (10.2)       | 13 (24.5)              | 21 (51.2)          |                      |        |
| Lymphatic permeation            |                |                        |                    |                      |        |
| Absent                          | 49 (100)       | 50 (94.3)              | 32 (78.0)          | .244                 | .0279  |
| Present                         | 0 (0)          | 3 (5.7)                | 9 (22.0)           |                      |        |
| Vascular invasion               |                |                        |                    |                      |        |
| Absent                          | 49 (100)       | 50 (94.3)              | 32 (78.0)          | .244                 | .0279  |
| Present                         | 0 (0.0)        | 3 (5.7)                | 9 (22.0)           |                      |        |

Note: Data are shown as number (%) among the number of patients in each group or median (range).

<sup>a</sup>P values represent ART-0 vs ART-low (left column) and ART-low vs. ART-high (right column).

index before treatment. In addition, in this study, posttreatment Ki-67 index was significantly lower in the ART-low group than in the ART-high group. This could be consistent with a previous report that the posttreatment Ki-67 index was a prognostic factor.<sup>23</sup>

Necrosis has been reported to be a predictor of prognosis in non-NAC breast cancer patients.<sup>24</sup> Therefore, we investigated the

effect of necrosis on prognosis. As in ART, the threshold value was determined by the ROC curve (0.89 mm<sup>2</sup>), and we classified into three groups (necrosis-0, 0 < necrosis ≤ 0.89, and necrosis > 0.89) to investigate the prognosis. However, the presence of necrosis and the area of necrosis had no effect on prognosis (Table 3, Figure S4).

FIGURE 5 Kaplan-Meier curves according to area of residual tumor (ART) in triple-negative breast cancer specimens. (A,B) Recurrence-free survival (RFS) curves (A) and overall survival (OS) curves (B) in the ART-0, ART-low, and ART-high groups. (C,D) RFS curves (C) and OS curves (D) in three groups of ARTN-α (ART-0 and ypN[–]), ARTN-β (ART-low and ypN[–]), and ARTN-γ (others). P values in RFS were P = 0.249 (α vs β), P < .001 (α vs γ), and P < .001 (β vs γ); P values in OS were P = 0.554 (α vs β), P < .001 (α vs γ), and P < .001 (β vs γ). (E,F) RFS curve (E) and OS curve (F) in three groups of ARTN-I (ART-0/low and ypN[–]), ARTN-II (ART-0/low, ypN[+] or ART-high, ypN[–]), and ARTN-III (ART-high and ypN[+]). P values in RFS were P = .020 (I vs II), P < .001 (I vs III), and P < .001 (II vs III); P values in OS were P = .041 (I vs II), P < .001 (I vs III), and P < .001 (II vs III)



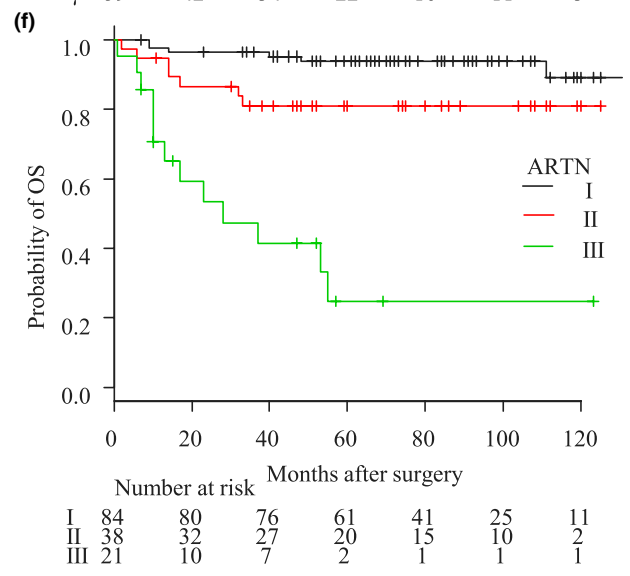
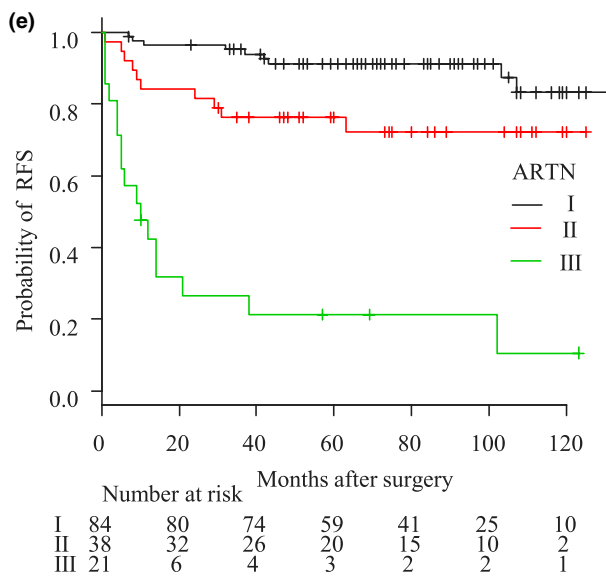
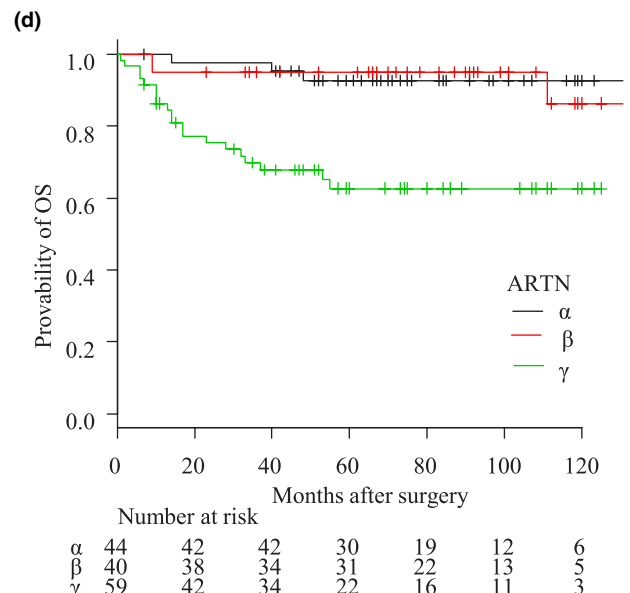
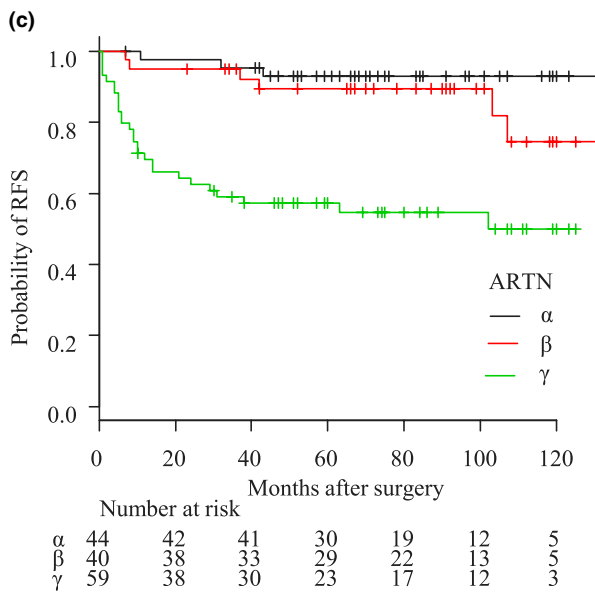
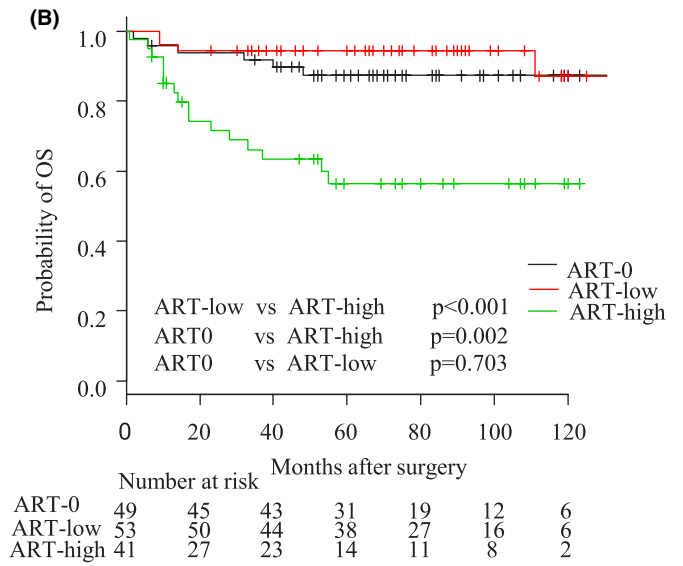
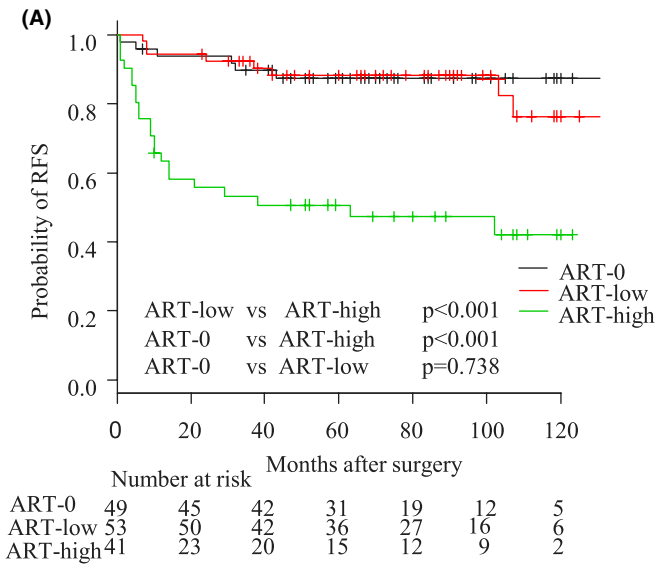


TABLE 3 Univariate and multivariate analyses of clinicopathological factors in 143 women with triple-negative breast cancer

| Variable                        | n   | Univariable analysis |              |         | Multivariable analysis |              |         |
|---------------------------------|-----|----------------------|--------------|---------|------------------------|--------------|---------|
|                                 |     | Hazard ratio         | 95% CI       | P value | Hazard ratio           | 95% CI       | P value |
| Age (y)                         |     |                      |              |         |                        |              |         |
| <40                             | 11  | Ref.                 |              |         |                        |              |         |
| ≥40                             | 132 | 1.315                | 0.316–5.477  | .707    |                        |              |         |
| cT                              |     |                      |              |         |                        |              |         |
| 1/2                             | 104 | Ref.                 |              |         | Ref.                   |              |         |
| 3/4                             | 39  | 1.409                | 1.011–1.409  | .043    | 1.209                  | 0.577–2.531  | .615    |
| Ki-67                           |     |                      |              |         |                        |              |         |
| <15                             | 16  | Ref.                 |              |         |                        |              |         |
| ≥15                             | 97  | 1.222                | 0.821–1.818  | .323    |                        |              |         |
| Pretreatment histological grade |     |                      |              |         |                        |              |         |
| 1/2                             | 65  | Ref.                 |              |         |                        |              |         |
| 3                               | 29  | 0.835                | 0.509–1.371  | .473    |                        |              |         |
| ypN                             |     |                      |              |         |                        |              |         |
| Negative                        | 104 | Ref.                 |              |         | Ref.                   |              |         |
| Positive                        | 39  | 6.549                | 3.328–12.890 | <.001   | 5.786                  | 2.664–12.560 | <.001   |
| Lymphatic permeation            |     |                      |              |         |                        |              |         |
| Absent                          | 131 | Ref.                 |              |         | Ref.                   |              |         |
| Present                         | 12  | 2.564                | 1.066–6.171  | .035    | 1.902                  | 0.706–5.124  | .204    |
| Vascular invasion               |     |                      |              |         |                        |              |         |
| Absent                          | 131 | Ref.                 |              |         | Ref.                   |              |         |
| Present                         | 12  | 3.129                | 1.295–7.561  | .011    | 1.458                  | 0.561–3.791  | .439    |
| Necrosis                        |     |                      |              |         |                        |              |         |
| Absent                          | 100 | Ref.                 |              |         |                        |              |         |
| Present                         | 43  | 1.415                | 0.7155–2.799 | .318    |                        |              |         |
| Radiotherapy                    |     |                      |              |         |                        |              |         |
| Absent                          | 32  | Ref.                 |              |         |                        |              |         |
| Present                         | 111 | 0.8126               | 0.382–1.729  | .590    |                        |              |         |
| ART                             |     |                      |              |         |                        |              |         |
| ART-0                           | 49  | Ref.                 |              |         | Ref.                   |              |         |
| ART-low                         | 53  | 1.202                | 0.417–3.466  | .733    | 0.707                  | 0.233–2.145  | .541    |
| ART-high                        | 41  | 5.798                | 2.346–14.330 | <.001   | 3.033                  | 1.081–8.511  | .035    |

Abbreviations: ART, area of residual tumor; CI, confidence interval; Ref., reference.

This study has two limitations: First, it is a retrospective study. However, as most postoperative non-pCR groups of TNBC are currently treated with capecitabine, it is difficult to carry out the same study in an observational study. Therefore, the best way to evaluate the usefulness of ART is to undertake a validation study in a multicenter trial. Second, the measurement of ART requires morphometric software, which is difficult to perform in some institutions. However, in pancreatic cancer, a semiquantitative assessment method of ART using the number of field views at 40× magnification has been proposed. This ART-based grading system has shown a high rate of agreement compared to other grading systems.<sup>25</sup> It

was noted in the same study that the area of a 40× field of view is 21.2 mm, so the cut-off value for this method in breast cancer would be seven fields of view. In fact, when we compared the hazard ratios at each cut-off value, we found that seven or eight fields of view were optimal (Figure S5). We plan to validate the utility of the optimal cut-off value for breast cancer with multicenter collaboration.

We concluded that ART is a candidate histopathological evaluation method for predicting the prognosis of TNBC patients treated with NAC. Furthermore, postoperative chemotherapy could be omitted in patients with ART-0 and ypN(-) (pathological complete response) and those with ART-low and ypN(-). Postoperative pathological

TABLE 4 Comparison of grading systems for triple-negative breast cancer

| A                   |     |                        |              |         |
|---------------------|-----|------------------------|--------------|---------|
| Variable            | n   | Univariate analysis    |              |         |
|                     |     | Hazard ratio           | 95% CI       | P value |
| JBCS grading system |     |                        |              |         |
| 0/1                 | 84  | 3.991                  | 1.660–9.593  | .002    |
| 2/3                 | 59  | Ref.                   |              |         |
| MPS                 |     |                        |              |         |
| 1/2/3               | 86  | 3.796                  | 1.579–9.124  | .003    |
| 4/5                 | 57  | Ref.                   |              |         |
| ypT                 |     |                        |              |         |
| ypT0/is/1           | 100 | Ref.                   |              |         |
| ypT2/3/4            | 43  | 3.18                   | 1.650–6.127  | <.001   |
| ART                 |     |                        |              |         |
| ART ≤ 136           | 102 | Ref.                   |              |         |
| ART > 136           | 41  | 5.24                   | 2.674–10.268 | <.001   |
| B                   |     |                        |              |         |
| Variable            | n   | Multivariable analysis |              |         |
|                     |     | Hazard ratio           | 95% CI       | P value |
| JBCS grading system |     |                        |              |         |
| 0/1                 | 84  | 2.211                  | 0.178–1.149  | .095    |
| 2/3                 | 59  | Ref.                   |              |         |
| MPS                 |     |                        |              |         |
| 1/2/3               | 86  | 2.019                  | 0.789–5.161  | .143    |
| 4/5                 | 57  | Ref.                   |              |         |
| ypT                 |     |                        |              |         |
| ypT0/is/1           | 100 | Ref.                   |              |         |
| ypT2/3/4            | 43  | 2.043                  | 1.033–4.041  | .04     |
| ART                 |     |                        |              |         |
| ART ≤ 136           | 102 | Ref.                   |              |         |
| ART > 136           | 41  | 3.812                  | 1.798–8.081  | <.001   |

Abbreviations: ART, area of residual tumor; CI, confidence interval; JBCS, Japanese Breast Cancer Society; MPS, Miller and Payne system; Ref., reference.

evaluation provides important information for predicting prognosis and determining future treatment strategies. In addition, postoperative pathological evaluation has been considered as a primary end-point in clinical trials in recent years because it is less expensive and has a shorter observation period than end-points such as disease-free survival and OS. In lung cancer, a major pathological response, defined as less than 10% residual tumor tissue, has been proposed as an alternative end-point.<sup>26</sup> In breast cancer, pCR has also been established as an alternative end-point.<sup>27,28</sup> In the near future, ART-based evaluation methods could also be indicated for this purpose.

## DISCLOSURE

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## REFERENCES

- Swisher SK, Vila J, Tucker SL, et al. Locoregional control according to breast cancer subtype and response to neoadjuvant chemotherapy in breast cancer patients undergoing breast-conserving therapy. *Ann Surg Oncol*. 2016;23:749-756.

2. Masuda N, Lee SJ, Ohtani S, et al. Adjuvant capecitabine for breast cancer after preoperative chemotherapy. *N Engl J Med*. 2017;376:2147-2159.
3. Sataloff DM, Mason BA, Prestipino AJ, Seinige UL, Lieber CP, Baloch Z. Pathologic response to induction chemotherapy in locally advanced carcinoma of the breast: a determinant of outcome. *J Am Coll Surg*. 1995;180:297-306.
4. Fisher B, Bryant J, Wolmark N, et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. *J Clin Oncol*. 1998;16:2672-2685.
5. Ogston KN, Miller ID, Payne S, et al. A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. *The Breast*. 2003;12:320-327.
6. 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;2001(30):96-102.
7. Mukai H, Arihiro K, Shimizu C, et al. Stratifying the outcome after neoadjuvant treatment using pathological response classification by the Japanese Breast Cancer Society. *Breast Cancer*. 2016;23:73-77.
8. Symmans WF, Peintinger F, Hatzis C, et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. *J Clin Oncol*. 2007;25:4414-4422.
9. Chollet P, Abrial C, Durando X, et al. A new prognostic classification after primary chemotherapy for breast cancer: residual disease in breast and nodes (RDBN). *Cancer J*. 2008;14:128-132.
10. Kobayashi K, Horii R, Ito Y, et al. Prognostic significance of histological therapeutic effect in preoperative chemotherapy for breast cancer. *Pathol Int*. 2016;66:8-14.
11. Carey LA, Metzger R, Dees EC, et al. American joint committee on cancer tumor-node-metastasis stage after neoadjuvant chemotherapy and breast cancer outcome. *JNCI: Journal of the National Cancer Institute*. 2005;97:1137-1142.
12. Yi M, Mittendorf EA, Cormier JN, et al. Novel staging system for predicting disease-specific survival in patients with breast cancer treated with surgery as the first intervention: Time to modify the current American joint committee on cancer staging system. *J Clin Oncol*. 2011;29:4654-4661.
13. Mittendorf EA, Jeruss JS, Tucker SL, et al. Validation of a novel staging system for disease-specific survival in patients with breast cancer treated with neoadjuvant chemotherapy. *J Clin Oncol*. 2011;29:1956-1962.
14. Von Minckwitz G, Untch M, Blohmer J-U, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. *J Clin Oncol*. 2012;30:1796-1804.
15. Cortazar P, Zhang L, Untch M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *The Lancet*. 2014;384:164-172.
16. Kojima M, Ishii G, Yamane Y, Nishizawa Y, Saito N, Ochiai A. Area of residual tumor beyond the muscular layer is a useful predictor of outcome in rectal cancer patients who receive preoperative chemoradiotherapy. *Pathol Int*. 2009;59:857-862.
17. Yamane Y, Ishii G, Goto K, et al. A novel histopathological evaluation method predicting the outcome of non-small cell lung cancer treated by neoadjuvant therapy: the prognostic importance of the area of residual tumor. *J Thorac Oncol*. 2010;5:49-55.
18. Okubo S, Kojima M, Matsuda Y, et al. Area of residual tumor (ART) can predict prognosis after post neoadjuvant therapy resection for pancreatic ductal adenocarcinoma. *Sci Rep*. 2019;9:17145.
19. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant*. 2013;48:452-458.
20. Fasching PA, Heusinger K, Haeberle L, et al. Ki67, chemotherapy response, and prognosis in breast cancer patients receiving neoadjuvant treatment. *BMC Cancer*. 2011;11:486.
21. Von Minckwitz G, Sinn H-P, Raab G, et al. Clinical response after two cycles compared to HER2, Ki-67, p53, and bcl-2 in independently predicting a pathological complete response after preoperative chemotherapy in patients with operable carcinoma of the breast. *Breast Cancer Res*. 2008;10:R30.
22. Jones RL, Salter J, A'Hern R, 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;119:315-323.
23. Guarneri V, Piacentini F, Ficarra G, et al. A prognostic model based on nodal status and Ki-67 predicts the risk of recurrence and death in breast cancer patients with residual disease after preoperative chemotherapy. *Ann Oncol*. 2009;20:1193-1198.
24. Gilchrist KW, Gray R, Fowble B, Tormey DC, Taylor SG. Tumor necrosis is a prognostic predictor for early recurrence and death in lymph node-positive breast cancer: a 10-year follow-up study of 728 Eastern Cooperative Oncology Group patients. *J Clin Oncol*. 1993;11:1929-1935.
25. Matsuda Y, Ohkubo S, Nakano-Narusawa Y, et al. Objective assessment of tumor regression in post-neoadjuvant therapy resections for pancreatic ductal adenocarcinoma: comparison of multiple tumor regression grading systems. *Sci Rep*. 2020;10:1-12.
26. Hellmann MD, Chaft JE, William WN Jr, et al. Pathological response after neoadjuvant chemotherapy in resectable non-small-cell lung cancers: proposal for the use of major pathological response as a surrogate endpoint. *Lancet Oncol*. 2014;15:e42-50.
27. Gianni L, Pienkowski T, Im Y-H, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2012;13:25-32.
28. Schmid P, Cortes J, Pusztai L, et al. Pembrolizumab for early triple-negative breast cancer. *N Engl J Med*. 2020;382:810-821.

## SUPPORTING INFORMATION

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