Original Study

BRCAness Predicts Resistance to Taxane-Containing Regimens in Triple Negative Breast Cancer During Neoadjuvant Chemotherapy☆

Sadako Akashi-Tanaka,[1] Chie Watanabe,[1] Tomoko Takamaru,[1] Takashi Kuwayama,[1] Murasaki Ikeda,[1] Hiroto Ohyama,[1] Miki Mori,[1] Reiko Yoshida,[1] Rikako Hashimoto,[1] Sawada Terumasa,[1] Katsutoshi Enokido,[1] Yuko Hirota,[2] Hiromi Okuyama,[3] Seigo Nakamura[1]

Abstract

We investigated BRCAness in the biopsy and surgical specimens from 73 patients with breast cancer, taken before and after taxane-containing neoadjuvant chemotherapy. All tumors that progressed on taxane-containing regimens had a poor prognosis; all had BRCAness and most were triple negative. Identifying BRCAness can help predict the response to taxane-containing regimens.

Background: To provide optimal treatment of heterogeneous triple negative breast cancer (TNBC), we need biomarkers that can predict the chemotherapy response. **Patients and Methods:** We retrospectively investigated BRCAness in 73 patients with breast cancer who had been treated with taxane- and/or anthracycline-based neoadjuvant chemotherapy (NAC). Using multiplex, ligation-dependent probe amplification on formalin-fixed core needle biopsy (CNB) specimens before NAC and surgical specimens after NAC. BRCAness status was assessed with the assessor unaware of the clinical information. **Results:** We obtained 45 CNB and 60 surgical specimens from the 73 patients. Of the 45 CNB specimens, 17 had BRCAness (38.6% of all subtypes). Of the 23 TNBC CNB specimens, 14 had BRCAness (61% of TNBC cases). The clinical response rates were significantly lower for BRCAness than for non-BRCAness tumors, both for all tumors (58.8% vs. 89.3%, $P = .03$) and for TNBC (50% vs. 100%, $P = .02$). All tumors that progressed with taxane therapy had BRCAness. Of the patients with TNBC, those with non-BRCAness cancer had pathologic complete responses significantly more often than did those with BRCAness tumors (77.8% vs. 14.3%, $P = .007$). After NAC, the clinical response rates were significant lower for BRCAness than for non-BRCAness tumors in all subtypes ($P = .002$) and in TNBC cases ($P = .008$). After a median follow-up of 26.4 months, 6 patients—all with BRCAness—had developed recurrence. Patients with BRCAness had shorter progression-free survival than did those with non- BRCAness ($P = .049$). **Conclusion:** Identifying BRCAness can help predict the response to taxane, and changing regimens for BRCAness TNBC might improve patient survival. A larger prospective study is needed to further clarify this issue.

Clinical Breast Cancer, Vol. 15, No. 1, 80-5 © 2015 The Authors. Published by Elsevier Inc. All rights reserved.
Keywords: Multiplex ligation-dependent probe amplification, Platinum salts, Predictive marker, Prognostic marker, Taxane resistance

Introduction

Triple negative breast cancer (TNBC) includes diverse histologic phenotypes and molecular profiles, with varying responses to therapy.[1] Approximately one third of patients with high-grade TNBC respond well to neoadjuvant chemotherapy (NAC) containing anthracycline and taxane and achieve a pathologic complete

[1]Department of Breast Surgical Oncology, Showa University School of Medicine, Tokyo, Japan
[2]Department of Pathology, Showa University School of Medicine, Tokyo, Japan
[3]Department of Pharmacy, Showa University School of Medicine, Tokyo, Japan

Submitted: May 3, 2014; Revised: Aug 12, 2014; Accepted: Aug 25, 2014; Epub: Sep 28, 2014

Address for correspondence: Sadako Akashi-Tanaka, MD, PhD, Department of Breast Surgical Oncology, Showa University School of Medicine, 1-5-8, Hatanodai, Shinagawa-ku, Tokyo 142-8666 Japan
E-mail contact: sakashi@med.showa-u.ac.jp

1526-8209/$ - see frontmatter © 2015 The Authors. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.clbc.2014.08.003

response (pCR), which is reportedly a surrogate marker for overall survival in TNBC.[2,3] However, approximately 20% of patients with TNBC will develop progression during NAC, especially those receiving taxane regimens, and have a very poor prognoses.[4,5] Diagnostic imaging studies and immunohistochemical and histologic studies cannot distinguish between resistant and sensitive TNBC tumors. Therefore, quick, accessible, and reproducible biomarkers are needed to identify the optimal chemotherapeutic regimens for patients with this heterogeneous disease.

Recent randomized trials have shown that adding carboplatin to anthracycline and taxane for NAC improves the pCR rates for TNBC; 2 meta-analyses found similar effects from adding platinum agents to NAC regimens.[6-9] However, adding carboplatin to standard NAC increases the incidence of adverse events, leading to greater rates of discontinuation and dose modification. Whether carboplatin should be added to, or substituted for, standard NAC regimens is unclear; thus, markers that can predict the response to standard NAC are needed.

"BRCAness" refers to some sporadic cancers that share phenotypic characteristics with tumors that carry BRCA1/2 mutations (BRCAMut), such as methylation of BRCA1/2 promoters and low BRCA1 gene expression.[10] Double-strand DNA breaks (DSBs) are repaired by homologous recombination, mediated by the products of BRCA1 and BRCA2 and by nonhomologous end-joining. Single-strand breaks are repaired by the base-excision repair pathway, which is regulated by poly-ADP ribose polymerase (PARP) 1 and by nucleotide exon repair mechanisms. Therefore, because BRCAMut tumors cannot repair DSBs induced by agents such as bifunctional alkylators and platinum salts, they are hypersensitive to DSB-inducing agents and probably to PARP inhibitors.[11-14] Assessment of BRCAness using array comparative genomic hybridization (aCGH) or multiplex ligation-dependent probe amplification (MLPA) has recently been described.[15] Patients with BRCAness tumors survive longer when treated intensively with alkylating agents as adjuvant chemotherapy.[15] Although germline mutations in BRCA1 and BRCA2 genes have been associated with $\leq$ 15% of TNBC cases,[16,17] MLPA assessments of BRCA status have indicated that these mutations are seen in approximately two thirds of TNBC cases.[18] The number of those who will benefit from targeted chemotherapy regimens and/or PARP inhibitors might be larger when using BRCAness, rather than BRCA1/2 mutation status, as the determinant.

Emerging preclinical and some clinical studies have indicated that BRCA-associated tumors tend to be resistant to taxanes.[16,17] Mammary tumors of BRCAMut$^+$ mice are resistant to doxorubicin and docetaxel but not to cisplatin.[16] An in vitro study has shown a BRCAMut$^+$ breast cancer cell line to be resistant to taxane.[1] BRCAMut$^+$ hormone receptor–positive metastatic breast cancer has been shown to be less sensitive to taxane.[17] However, to our knowledge, the association between taxane response and BRCAness has not been previously reported.

The present study investigated whether BRCAness can predict the response to taxane treatment in patients with breast cancer treated with NAC.

Patients and Methods

Patients

All the patients who received NAC with either taxane and/or anthracycline for primary breast cancer from October 2010 to March 2013 at Showa University Hospital Breast Center were included in the present retrospective study. Most of these patients had been in randomized controlled trials comparing the efficacy and feasibility of docetaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide (FEC) every 3 weeks or weekly albumin-bound paclitaxel (nab-paclitaxel) followed by FEC as NAC for patients with human epidermal growth factor receptor 2 (HER2)–negative breast cancer. Before these trials had started, the regimen administered was docetaxel and cyclophosphamide every 3 weeks.

For HER2$^+$ tumors, the regimen was FEC followed by docetaxel and trastuzumab. Relevant clinicopathologic information was collected from our database and medical records. Clinical responses were determined by ultrasonography and magnetic resonance imaging using the Response Evaluation Criteria in Solid Tumors,[19] with imaging performed before NAC and at the end of the first and second cycles. The clinical response rate (cRR) was defined as the sum of the clinical complete and partial responses (PRs). In patients whose tumors progressed, the regimen was stopped, and either surgery performed or a second-line regimen substituted.

The patients underwent surgery approximately 1 month after completing the last NAC cycle. The surgical procedures were determined according to the diagnostic imaging findings after NAC completion. Sentinel lymph node biopsy was performed in patients whose lymph nodes had been clinically negative before NAC.

The institutional review board of our university approved present the study.

Pathology

The tumor subtypes were routinely determined immunohistochemically before NAC, using core needle biopsy (CNB) specimens. The cancer specimens were defined as HER2$^+$ when HER2 immunohistochemical staining was 3+ or fluorescence in situ hybridization (FISH) showed HER2 gene amplification. Estrogen receptor (ER) and progesterone receptor (PgR) positivity was defined as $\geq$ 1% of tumor cells staining positive for ER or PgR. A pCR was defined as complete remission of the invasive components of cancer in the breast.[20]

MLPA Method

BRCAness was determined by examination of formalin-fixed, paraffin-embedded (FFPE) CNB specimens taken before NAC and surgical specimens taken after NAC. DNA was isolated from the tumor tissue using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) after macrodissection. Classification of BRCAness was performed using MLPA with the Probemix P376-B2 BRCA1ness (MRC-Holland, Amsterdam, The Netherlands), as previously reported by Oonk et al.[13] MLPA was performed at Falco Biosystems (Kyoto, Japan) as a part of collaborative research and according to the manufacturer's instructions. For each sample, the relative copy number ratios for the 38 target-specific probes, compared with the reference samples of human genomic DNA (Promega, Madison, WI), were calculated using the Coffalyser.Net software and were used for the prediction analysis for microarrays, with the training set generated by MRC-Holland. Each sample was analyzed twice. The average scores were used for this analysis. BRCAness status was analyzed by experienced laboratory scientists who were unaware of the

patients' clinical information. The cutoff ratio for BRCAness positivity was 0.4.

BRCA1/2 Germline Mutation

Genetic counseling was recommended for patients suspected of having BRCA germline mutations, in accordance with the National Comprehensive Cancer Network guidelines. One half of them underwent genetic testing. BRCA1/2 mutation analysis was performed at Falco Biosystems (Kyoto, Japan) using the direct sequencing method on patient blood samples. If this initial analysis did not detect a mutation, the sample was checked again for BRCA1/2 genetic rearrangements using MLPA.

Statistical Analysis

Fisher's exact test was used to assess the differences between the BRCAness and non-BRCAness groups. Student's *t* test was used to assess the differences between the BRCAness and non-BRCAness groups for the Ki-67 index. The log-rank test was used to evaluate the differences in relapse-free survival. The software used was EZR on R[21] for Fisher's exact test and SPSS for the log-rank test.

Results

Of the 73 patients who underwent NAC, surgical specimens were available from 60 patients and CNB specimens from 45 patients for BRCAness analysis (Table 1). In 13 patients who had a pCR, BRCAness could not be measured on the surgical specimens. Also, 28 CNB specimens were not available, because the CNBs had been performed by the patients' family doctor. The patients' overall mean age at the diagnosis of breast cancer was 42.0 years (range, 27-75 years). Nine patients underwent genetic testing for BRCA1/2 germline mutation after genetic counseling; 5 patients carrying the BRCA1 germline mutation and 2 with the BRCA2 mutation were identified among the 73 patients. Of these 5 patients, 4 with the BRCA1 germline mutation and 1 with the BRCA2 mutation were BRCAness positive.

Of the 45 CNB specimens of all tumor subtypes, 17 (23.3%) were BRCAness positive. Of the 23 CNB specimens with TNBC, 14 (60.9%) were BRCAness positive. The other 3 BRCAness tumors without TNBC included 2 HER2-enriched tumors and 1 ER^+ tumor. One tumor that was $HER2^+$ before NAC had changed to TNBC after NAC. Another $HER2^+$ tumor was immunohistochemically 3+ but had been FISH-negative before NAC. The tumor tested positive after NAC. The only ER^+ tumor remained ER^+ after NAC.

We analyzed the association between BRCAness found in the CNB specimen and the cRR for taxane-containing regimens (Table 2). The cRR was significantly lower for BRCAness tumors of all subtypes (58.8%) than for non-BRCAness tumors (89.3%; $P = .027$) and, more strikingly so, for BRCAness TNBC (50%) than for non-BRCAness TNBC (100%; $P = .019$). All the non-BRCAness TNBC cases responded well to taxane regimens.

Five patients experienced progressive disease (PD) during taxane-containing NAC (Table 3), including 4 with BRCAness TNBC and 1 with a mucinous ER^+/PgR^+ carcinoma. CK5/6 and epidermal growth factor receptor were not effective in predicting the response to taxane.

Table 1 Relevant Patient and Tumor Characteristics

Variable	All NAC Patients (n = 73)	CNB Available (n = 45)
Age (years)	42.0 (27-75)	42.3 (27-73)
Tumor size before NAC		
T1	10 (13.7)	5 (11.1)
T2	43 (58.9)	28 (62.2)
T3	15 (20.5)	9 (20.0)
T4b	5 (6.8)	3 (6.7)
Histologic type before NAC		
IDC	68 (93.2)	40 (88.9)
ILC	2 (2.7)	2 (4.4)
Apocrine	1 (1.4)	1 (2.2)
Mucinous	2 (2.7)	2 (4.4)
Subtype before NAC		
Triple negative	26 (35.6)	23 (51.1)
$ER^-/HER2^+$	13 (17.8)	7 (15.6)
$ER^+/HER2^+$	7 (9.6)	2 (4.4)
$ER^+/HER2^-$	27 (37.0)	13 (28.9)
Regimen		
Taxane + anthracycline	48 (65.8)	32 (72.7)
Taxane	12 (16.4)	10 (22.7)
Taxane + anthracycline + trastuzumab	13 (17.8)	3 (4.5)
Clinical response		
CR	7 (9.6)	7 (15.9)
PR	47 (64.4)	28 (61.4)
SD	14 (19.2)	5 (11.4)
PD	5 (6.8)	5 (11.4)
Pathologic response		
pCR	11 (15.1)	11 (25.0)
Other	62 (84.9)	34 (75.0)
Pathologic nodal status		
N0	46 (61.3)	32 (71.1)
N1-N3	18 (24.7)	8 (17.8)
N4 or greater	9 (12.3)	5 (11.1)

Data presented as mean (range) or n (%).
Abbreviations: CNB = core needle biopsy; CR = complete response; ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma; NAC = neoadjuvant chemotherapy; pCR = pathologic complete response; PD = progressive disease; PR = partial response; SD = stable disease.

Patients with non-BRCAness TNBC achieved pCRs significantly more often (77.8%) than did those with BRCAness TNBC (14.3%; $P = .0066$; Table 4). Before NAC, the patients with BRCAness or non-BRCAness TNBC did not differ significantly in any other clinicopathologic factor.

Of the 60 surgical specimens taken after NAC, 9 (15.0%) were BRCAness positive. Analysis of the association between BRCAness subtype and the cRR after taxane-containing regimens showed that, for all subtypes, the cRR was significantly lower for BRCAness tumors (22.2%) than for non-BRCAness tumors (78.4%; $P = .002$) and more so for BRCAness TNBC tumors (14.3%) than for non-BRCAness TNBC tumors (88.9%; $P = .008$).

Table 2 Relationships Between BRCAness Before Neoadjuvant Chemotherapy and Clinical Responses to Taxane for All Subtypes and for Triple Negative Breast Cancer

Clinical Response	BRCAness (%)	Non-BRCAness (%)	*P* Value (Fisher's Exact Test)
All			.027
CR+PR	10 (58.8)	25 (89.3)	
SD+PD	7 (41.2)	3 (10.7%)	
TNBC			.019
CR+PR	7 (50)	9 (100)	
SD+PD	7 (50)	0 (0)	

Abbreviations: CR = complete response; PD = progressive disease; PR = partial response; SD = stable disease; TNBC = triple negative breast cancer.

The median follow-up duration from the initiation of NAC was 26.4 months. Of the 26 patients with TNBC before NAC, 6 developed a recurrence, including 3 with locoregional recurrence and 3 with distant metastases (1 patient each with brain, lung, or liver metastases). All 3 locoregional recurrences had developed after radical mastectomy for BRCAness TNBC. The patients with BRCAness had worse progression-free survival than those with non-BRCAness (58% vs. 100%, $P = .049$).

Figure 1 shows the BRCAness changes in TNBC after NAC. Of the 14 BRCAness TNBC tumors before NAC, 5 remained BRCAness positive after NAC. Of these 5 patients, 3 had PD and 2 stable disease; 3 developed a recurrence. Of the 14 BRCAness TNBCs before NAC, 7 tested negative after NAC. Their responses to taxane varied from a PR to PD. In contrast, of 9 tumors that were non-BRCAness before NAC, 7 (77.8%) had achieved a pCR and 2 had a clinical PR.

Discussion

The results of the present study indicate that BRCAness tumors have a significantly poorer response to taxane regimens than do non-BRCAness tumors. Originally, BRCAness was identified by comparing BRCAMut tumors with sporadic TNBC tumors using the aCGH method. However, MLPA probes are now commercially available. Adjuvant therapy with high-dose, platinum-based, alkylating agents is reportedly more effective for BRCAness tumors (according to aCGH) than conventional chemotherapy; this has not been true for non-BRCAness tumors.[11] The assessment of BRCAness using MLPA and aCGH is reportedly concordant (accuracy 94%) and also predicts similar survival benefits with intensive alkylating agent chemotherapy.[15] However, with conventional dose anthracycline chemotherapy, the prognoses of BRCAness and non-BRCAness tumors are similar.[13] Patients with BRCAness tumors have substantially better outcomes after adjuvant DSB-inducing chemotherapy.[11] Together with our findings, these results imply that administering platinum salts according to BRCAness status in patients with TNBC will be preferable to administering them to all TNBC patients. Our recommended treatment strategy is that patients with BRCAness tumors receive platinum- and anthracycline-based chemotherapy and that patients with non-BRCAness TNBC receive standard taxane- and

Table 4 Relationships Between BRCAness Before, and Pathologic Responses to, Neoadjuvant Chemotherapy for Triple Negative Breast Cancer

Variable	BRCAness (n = 14)	Non-BRCAness (n = 9)	*P* Value
Average age (years)	46 (27-62)	48 (37-59)	NS
Tumor size before NAC			NS
T1	1 (7.1)	2 (22.2)	
T2	11 (78.6)	7 (77.8)	
T3	1 (7.1)	0 (0)	
T4b	1 (7.1)	0 (0)	
Histologic grade			NS
1-2	5 (35.7)	3 (33.3)	
3	8 (57.1)	5 (55.6)	
ND	1	2	
Pathologic response			.0066
pCR	2 (14.3)	7 (77.8)	
Other	12 (85.7)	2 (22.2)	
Pathologic nodal status			NS
N0	9 (64.3)	7 (77.8)	
N+	5 (35.7)	2 (22.2)	
Average Ki-67 (%)	66.0	58.1	NS
Recurrence			NS
Yes	5 (35.7)	0 (0)	
No	9 (64.3)	9 (100)	

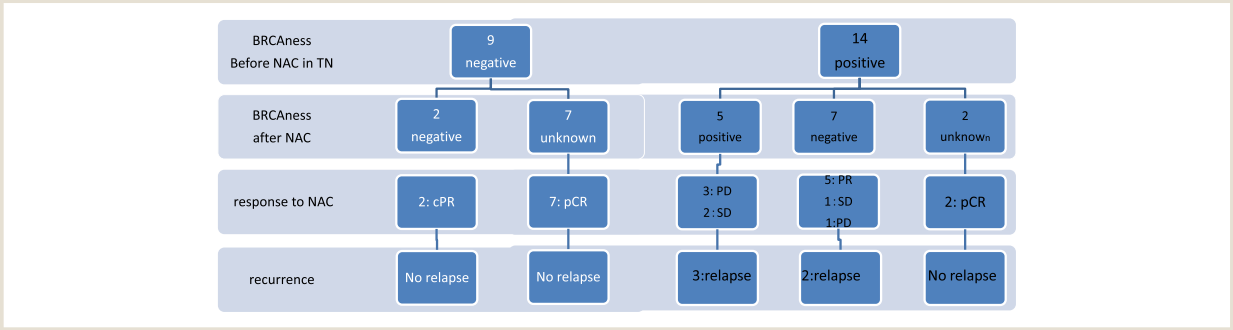
Data presented as average (range) or n (%).
Abbreviations: NAC = neoadjuvant chemotherapy; ND = not determined; NS = not significant; pCR = pathologic complete response.

Table 3 Characteristics of Tumors That Developed Progressive Disease During Taxane-Based Neoadjuvant Chemotherapy

Pt. No.	NAC Response	Germline Mutation	BRCAness	ER	PgR	HER2	Ki-67	EGFR	CK-56	Pathologic Response
1	PD @ Doce 1 cycle (FEC)	BRCA2	+	−	−	1	80-90	+	+	Poor response
2	PD @ Doce 1 cycle	NP	+	−	−	0	60-70	+	−	No response
3	PD @ Doce 2 cycle (FEC)	NP	+	−	−	0	60-70	+	−	No response
4	PD @ nabPTX 7 cycle (FEC)	BRCA1	+	−	−	0	50-60	−	−	Poor response
5	PD @ TC 3 cycle	NP	−	+	+	1	5	NP	NP	No response

Abbreviations: + = positive; − = negative; Doce = docetaxel; EGFR = epidermal growth factor receptor; ER = estrogen receptor; FEC = 5-fluorouracil, epirubicin, cyclophosphamide; HER2 = human epidermal growth factor receptor 2; nabPTX = albumin-bound paclitaxel; NAC = neoadjuvant chemotherapy; NP = not performed; PD = progressive disease; PgR = progesterone receptor; Pt. No. = patient number; TC = docetaxel, cyclophosphamide.

Figure 1 Changes in BRCAness Status After Neoadjuvant Chemotherapy in Triple Negative Breast Cancer and Relationship to Clinical Response



Abbreviations: CR = complete response; NAC = neoadjuvant chemotherapy; ND = no data; NS = not significant.

anthracycline-based chemotherapy. Adding a PARP inhibitor, for both patients with BRCA1 germline mutations and those with BRCAness, might improve survival[12] and warrants additional study.

The ability to predict resistance to taxane treatment by BRCAness status was shown in all our patients, regardless of subtype, but especially in the TNBC subgroup. The reported rates of BRCAness assessed using the aCGH and MLPA methods were 18% for all subtypes and 69% for TNBC.[11,22] Approximately two thirds of TNBC tumors in the present study were BRCAness positive, but only a few non-TNBC tumors were BRCAness positive, corresponding with the results from previous reports. Therefore, we recommend assessing BRCAness status only for patients with TNBC.

The mechanisms for resistance to taxane by BRCAness tumors have not yet been established. Intact BRCA1 function might play an important role in the optimal response to taxane-based therapy.[23] A BRCA1-induced increase in the c-Jun N-terminal kinase pathway causes apoptosis in BRCA1-expressing cells treated with paclitaxel.[24,25]

Tumors with low BRCA1 expression, demonstrated by immunohistochemistry, have had shorter times to progression when treated with taxane-containing regimens.[26] However, this finding has not been confirmed by other investigators, possibly because of the poor reproducibility of the BRCA1 antibody assays. A homologous recombination deficiency assay reported in 2013,[27] which performs genome-wide, single-nucleotide polymorphism analysis using Affymetrix molecular inversion probe arrays of DNA sequencing, is also effective for selecting likely responders to neoadjuvant carboplatin, gemcitabine, and iniparib.

Reportedly, the pCR rate after a NAC regimen of dose-dense cyclophosphamide and doxorubicin was significantly greater in BRCA1-mutated tumors (63%) than in non-BRCA1–mutated tumors (33%). The pCR rate also tended to be greater in BRCAness than in non-BRCAness tumors (35% vs. 21%).[22] However, these investigators also reported that the recurrence rates after adjuvant chemotherapy with anthracycline-based regimens did not differ between these groups. In our study, patients with BRCAness tumors treated with taxane and/or anthracycline had a poor prognosis, developing both PD and PRs. These discrepancies in outcomes likely reflect the different regimens used.

Paluch-Shimon et al[28] reported that BRCA1/2-associated TNBC had a better pCR rate than TNBC in noncarriers (61% vs. 39%; $P = .007$) after dose-dense NAC with an anthracycline and a taxane, opposite the results in our study. They also reported that the pCR was not associated with the long-term outcome in BRCA1/2-associated TNBC, unlike non-BRCA–associated TNBC, probably owing to enrichment of the cancer stem cells in BRCA1/2 tumors.[28] In our study, all 6 patients with tumor relapse had BRCAness-positive tumors and no pCR response. In contrast, patients with a pCR had a better prognosis. Only 7 of our patients who had BRCA1/2 germline mutations, 4 of whom (57%) achieved a pCR, and none of whom relapsed. Two of the patients with BRCA1/2-associated breast cancer who did not achieve a pCR developed a relapse. In our small series, a pCR also seemed to be associated with better long-term outcomes in patients with BRCA1/2-associated breast cancer. The characteristic differences in terms of chemosensitivity and cancer stem cells among BRCA1/2-associated TNBC cases and BRCAness cases should be investigated further.

In the present study, we used a cutoff ratio for BRCAness of 0.4. However, in the original report, the cutoff point was 0.5.[11] The scores in about 75% of the BRCAness tumors were > 0.7, but approximately 80% of the non-BRCAness tumors scored < 0.2. Thus, these 2 categories are easy to differentiate. The score of 1 patient with PD after 2 cycles of docetaxel was 0.42 before NAC and 0.86 after NAC. In no other patient did the BRCAness status change from negative to positive after NAC. Therefore, we applied this cutoff point. A larger scale study is needed to clarify the appropriate cutoff point.

The present study had some limitations. The first was that it was a small retrospective analysis. Retrospective validation studies using NAC cohorts from other hospitals are ongoing. A larger prospective study is needed to validate our findings. Second, some data concerning BRCAness before NAC were unavailable, because we did not perform new biopsies in patients who had been already diagnosed with breast cancer at other hospitals.

Conclusion

Identifying the BRCAness status can help predict the response to taxane, and changing regimens for BRCAness TNBC might improve patient survival. A larger prospective study is needed to further clarify this issue.

Clinical Practice Points

- Approximately one fifth of TNBC tumors progress during NAC, especially those treated with taxane-containing regimens.
- Adding platinum salts to standard NAC regimens significantly improves the pCR rate in patients with TNBC. Although whether platinum salts should be added to, or substituted for, the standard regimen is controversial.
- We found that most patients with non-BRCAness TNBC achieved a pCR rate using the standard regimen; however, patients with BRCAness TNBC were more likely to develop PD and have a worse prognosis.
- Adjuvant therapy with high-dose, platinum-based alkylating agents is reportedly more effective than conventional chemotherapy for BRCAness tumors but not for non-BRCAness tumors. Therefore, platinum salts for TNBC should be selected according to the BRCAness status rather than adding it to the regimens of all patients with TNBC.
- The method we used is clinically feasible and requires only commercially available MLPA probes.
- In the future, this biomarker might also assist in the selection of patients with TNBC to receive a PARP inhibitor.

Acknowledgments

The assays for BRCAness were performed by Falco Biosystems (Kyoto, Japan) at no cost under a collaborative study contract. The present study was partly supported by a grant for the Third Comprehensive Strategy for Cancer from the Ministry of Health, Labour and Welfare, H23-cancer-004, Japan.

Disclosure

The authors have stated that they have no conflicts of interest.

References

1. Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest* 2011; 121:2750-67.
2. Cortazar P, Zhang L, Untch M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet* 2014; 384:164-72.
3. von Minckwitz G, Untch M, Blohmer JU, 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.
4. Iwata H, Sato N, Masuda N, et al. Docetaxel followed by fluorouracil/epirubicin/cyclophosphamide as neoadjuvant chemotherapy for patients with primary breast cancer. *Jpn J Clin Oncol* 2011; 41:867-75.
5. Shien T, Yoshida M, Hojo T, et al. Clinicopathologic features of primary breast cancer resisting primary systemic therapy. *Jpn J Breast Cancer* 2008; 23:49-53.
6. Von Minckwitz G, Schneeweiss A, Salat C, et al. Addition of Carboplatin Beneficial in Neoadjuvant Treatment of Triple-NegativeBreast Cancer. *J Clin Oncol (Proc ASCO)* 2014, 1004.
7. Sikov WN, Berry DA, Perou CM, et al. The impact of addition of carboplatin and/or bevacizumab to neoadjuvant weekly paclitaxel followed by dose-dense AC on pathologic complete response rate in triple -negative breast cancer: CALGB 40603 (Alliance). *J Clin Oncol* Published Ahead of Print on August 4, 2014 as http://dx.doi.org/10.1200/JCO.2014.57.0572.
8. Petrelli F, Coinu A, Borgonovo K, et al. The value of platinum agents as neoadjuvant chemotherapy in triple-negative breast cancers: a systematic review and meta-analysis. *Breast Cancer Res Treat* 2014; 144:223-32.
9. Liu M, Mo QG, Wei CY, et al. Platinum-based chemotherapy in triple-negative breast cancer: a meta-analysis. *Oncol Lett* 2013; 5:983-91.
10. Turner N, Tutt A, Ashworth A. Hallmarks of "BRCAness" in sporadic cancers. *Nat Rev Cancer* 2004; 4:814-9.
11. Vollebergh MA, Lips EH, Nederlof PM, et al. An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients. *Ann Oncol* 2011; 22:1561-70.
12. Lee JM, Ledermann JA, Kohn EC. PARP Inhibitors for BRCA1/2 mutation-associated and BRCA-like malignancies. *Ann Oncol* 2014; 25:32-40.
13. Oonk AM, van Rijn C, Smits MM, et al. Clinical correlates of "BRCAness" in triple-negative breast cancer of patients receiving adjuvant chemotherapy. *Ann Oncol* 2012; 23:2301-5.
14. Chalasani P, Livingston R. Differential chemotherapeutic sensitivity for breast tumors with "BRCAness": a review. *Oncologist* 2013; 18:909-16.
15. Lips EH, Laddach N, Savola SP, et al. Quantitative copy number analysis by multiplex ligation-dependent probe amplification (MLPA) of BRCA1-associated breast cancer regions identifies BRCAness. *Breast Cancer Res* 2011; 13:R107.
16. Rottenberg S, Nygren AO, Pajic M, et al. Selective induction of chemotherapy resistance of mammary tumors in a conditional mouse model for hereditary breast cancer. *Proc Natl Acad Sci U S A* 2007; 104:12117-22.
17. Kriege M, Jager A, Hooning MJ, et al. The efficacy of taxane chemotherapy for metastatic breast cancer in BRCA1 and BRCA2 mutation carriers. *Cancer* 2012; 118:899-907.
18. Rummel S, Varner E, Shriver CD, et al. Evaluation of BRCA1 mutations in an unselected patient population with triple-negative breast cancer. *Breast Cancer Res Treat* 2013; 137:119-25.
19. Eisenhauer EA, Bogaerts J, Schwartz LH, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; 45:228-47.
20. Shigenaga R, Akashi-Tanaka S. [Comparison among Japanese general rules for clinical and pathological recording of breast cancer, 16th ed and UICC TNM classification, 7th ed]. *Nihon Rinsho* 2012; 70(suppl 7):191-4.
21. Kanda Y. Investigation of the freely available easy-to-use software "EZR" for medical statistics. *Bone Marrow Transplant* 2013; 48:452-8.
22. Lips EH, Mulder L, Oonk A, et al. Triple-negative breast cancer: BRCAness and concordance of clinical features with BRCA1-mutation carriers. *Br J Cancer* 2013; 108:2172-7.
23. Sung M, Giannakakou P. BRCA1 regulates microtubule dynamics and taxane-induced apoptotic cell signaling. *Oncogene* 2014; 33:1418-28.
24. Lafarge S, Sylvain V, Ferrara M, et al. Inhibition of BRCA1 leads to increased chemoresistance to microtubule-interfering agents, an effect that involves the JNK pathway. *Oncogene* 2001; 20:6597-606.
25. Chabalier C, Lamare C, Racca C, et al. BRCA1 downregulation leads to premature inactivation of spindle checkpoint and confers paclitaxel resistance. *Cell Cycle* 2006; 5:1001-7.
26. Kurebayashi J, Yamamoto Y, Kurosumi M, et al. Loss of BRCA1 expression may predict shorter time-to-progression in metastatic breast cancer patients treated with taxanes. *Anticancer Res* 2006; 26:695-701.
27. Telli M, Jensen KC, Kurian AW, et al. PrECOG 0105: Final efficacy results from a phase II study of gemcitabine (G) and carboplatin (C) plus iniparib (BSI-201) as neoadjuvant therapy for triple-negative (TN) and BRCA1/2 mutation-associated breast cancer ASCO proceedings. 2013, Abstract PD 09-04.
28. Paluch-Shimon S, Friedman E, Berger R, et al. Does pathologic complete response predict for outcome in BRCA mutation carriers with triple-negative breast cancer? *J Clin Oncol* 2014; 32(suppl):5s (abstract 1023).