Excerpta Medica

The American Journal of Surgery

The American Journal of Surgery 191 (2006) 225–229
Scientific papers–International

Sentinel lymph node biopsy examination for breast cancer patients with clinically negative axillary lymph nodes after neoadjuvant chemotherapy

Takayuki Kinoshita, M.D.[a,*], Miyuki Takasugi, M.D.[a], Eriko Iwamoto, M.D.[a], Sadako Akashi-Tanaka, M.D.[a], Takashi Fukutomi, M.D.[a], Shoji Terui, M.D.[b]

[a]*Division of Surgical Oncology, National Cancer Center Hospital, 5-1-1, Tsukiji Chuo-ku, Tokyo 104-0045, Japan*
[b]*Division of Nuclear Medicine, National Cancer Center Hospital, Tsukiji Chuo-ku, Tokyo, Japan*

Manuscript received February 10, 2005; revised manuscript June 22, 2005

Abstract

Background: The feasibility and accuracy of sentinel lymph node (SLN) biopsy examination for breast cancer patients with clinically node-negative breast cancer after neoadjuvant chemotherapy (NAC) have been investigated under the administration of a radiocolloid imaging agent injected intradermally over a tumor. In addition, conditions that may affect SLN biopsy detection and false-negative rates with respect to clinical tumor response and clinical nodal status before NAC were analyzed.

Methods: Seventy-seven patients with stages II and III breast cancer previously treated with NAC were enrolled in the study. All patients were clinically node negative after NAC. The patients then underwent SLN biopsy examination, which involved a combination of intradermal injection over the tumor of radiocolloid and a subareolar injection of blue dye. This was followed by standard level I/II axillary lymph node dissection.

Results: The SLN could be identified in 72 of 77 patients (identification rate, 93.5%). In 69 of 72 patients (95.8%) the SLN accurately predicted the axillary status. Three patients had a false-negative SLN biopsy examination result, resulting in a false-negative rate of 11.1% (3 of 27). The SLN identification rate tended to be higher, although not statistically significantly, among patients who had clinically negative axillary lymph nodes before NAC (97.6%; 41 of 42). This is in comparison with patients who had a positive axillary lymph node before NAC (88.6%; 31 of 35).

Conclusions: The SLN identification rate and false-negative rate were similar to those in nonneoadjuvant studies. The SLN biopsy examination accurately predicted metastatic disease in the axilla of patients with tumor response after NAC and clinical nodal status before NAC. This diagnostic technique, using an intradermal injection of radiocolloid, may provide treatment guidance for patients after NAC.

Keywords: Sentinel node biopsy; Neoadjuvant chemotherapy; Clinically node negative; Intradermal injection

Currently, the status of the axillary lymph nodes remains the most important prognostic indicator for breast cancer and helps the physician in guiding adjuvant therapy. More than 40 peer-reviewed pilot studies published between 1993 and 1999 have established the validity of sentinel lymph node (SLN) biopsy examination technique for clinically node-negative breast cancer [1], and the SLN biopsy procedure has become the standard of care for axillary staging in these patients.

Recent studies report identification rates of more than 90%, with false-negative rates ranging from 2% to 10% [2,3]. To ensure a high SLN identification rate and a low false-negative rate, some relative contraindications for SLN biopsy examination have been established: these include T3 or T4 tumors, multicentric or multifocal lesions, a large biopsy cavity, previous axillary surgery, previous chest-wall irradiation, and neoadjuvant chemotherapy (NAC) [4,5].

The application of SLN biopsy examination in NAC-treated patients may, as in nonneoadjuvant chemotherapy groups, identify patients who do not necessarily require an axillary lymph node dissection (ALND). Several studies

* Corresponding author. Tel.: +81-3-3542-2511; fax: +81-3-3542-3815.
E-mail address: takinosh@ncc.go.jp

0002-9610/06/$ – see front matter
doi:10.1016/j.amjsurg.2005.06.049

Table 1
Patient demographics

	Number of patients
Age, y	
Mean	51.1
Range	27–75
Clinical tumor size, cm*	
Mean	4.82
Range	2.7–12
Tumor classification*	
T2	50 (65.0%)
T3	24 (31.2%)
T4	3 (3.8%)
Lymph node status*	
N0	42 (54.5%)
N1	28 (36.4%)
N2	7 (9.1%)
Tumor type	
Invasive ductal	74 (96.1%)
Invasive lobular	3 (3.9%)
Type of NAC	
FEC plus paclitaxel	73 (94.9%)
Paclitaxel alone	4 (5.1%)
Clinical response of the tumor	
CR	41 (53.2%)
PR	28 (36.4%)
SD	8 (10.4%)
Pathologic response of the tumor	
pCR	17 (22.1%)
pINV	60 (77.9%)
Pathologic nodal status	
Negative	47 (61.0%)
Positive	30 (39.0%)

CR = complete response; FEC = fluorouracil/epirubicin/cyclophosphamide; PR = partial response; SD = stable disease; pCR = pathologic complete response; pINV = pathologic invasive.
* Before NAC.

have evaluated the use of SLN biopsy examination in patients with breast cancer after NAC but results are varied and inconclusive [6–14].

Recently, several studies have shown the feasibility and accuracy of SLN biopsy examination using peritumoral injection of radiocolloid for patients with NAC-treated breast cancer. However, false-negative rates varied considerably among these studies [6–13]. It is possible that tumor response to chemotherapy may alter or interrupt the lymphatic drainage, thus causing the lower SLN identification rates and higher false-negative rates as opposed to nonneoadjuvant studies. Our hypothesis is that the lymphatic flow within the skin lesion overlying the tumor is less damaged by the chemotherapy than that in the parenchyma surrounding the tumor, except in T4 tumors. Thus, the usefulness of SLN biopsy examination with intradermal injection of radiocolloid for patients with NAC-treated breast cancer has yet to be established.

The aim of this study was to determine the feasibility and accuracy of the SLN biopsy procedure using intradermal injection of radiocolloid over the tumor in clinically node-negative NAC-treated breast cancer patients.

Methods

Between May 2003 and January 2005, 77 patients with T2-4N0-2 breast cancer underwent NAC with SLN biopsy examination plus ALND performed by a single surgeon. The pathologic diagnosis was established by core needle biopsy examination in all patients.

Patients younger than 65 years of age received 4 cycles of 5-fluorouracil (500 mg/m^2)/epirubicin (100 mg/m^2)/cyclophosphamide (500 mg/m^2) plus 12 weekly cycles of paclitaxel (80 mg/m^2), and patients older than 65 years of age received 12 weekly cycles of paclitaxel (80 mg/m^2) alone. After NAC, we enrolled the 77 clinically node-negative patients in this study.

Lymphatic mapping was performed using a 3-mL combination of blue dye (Patent blue V; TOC Ltd, Tokyo, Japan) and 30 to 80 MBq of technetium-99m–labeled Phytate (Daiichi RI Laboratory, Ltd, Tokyo, Japan). The day before surgery, the radiotracer was injected intradermally into the area overlying the tumor, and blue dye was injected into the subareolar site intraoperatively. For nonpalpable lesions, injections were performed under mammographic or ultrasonic needle localization. Sentinel lymph nodes were identified as being stained blue, radioactive, or both. The SLN biopsy procedure then was followed by a standard level I/II ALND.

All sentinel nodes were evaluated histologically by submitting each node as a 3-mm to 5-mm serial section stained with hematoxylin-eosin. Lymph nodes submitted as part of the axillary dissection were totally submitted and evaluated using standard hematoxylin-eosin staining.

Results

Patient characteristics, type of chemotherapy, clinical response of the tumor, and pathologic findings are summarized in Table 1. All patients underwent breast-conserving therapy or mastectomy and were clinically node negative at the time of surgery.

As shown in Table 2, the overall SLN identification rate was 93.5% (72 of 77). Of the 72 patients in whom an SLN could be identified, 24 (33.3%) had positive SLNs. Within

Table 2
Results of sentinel node biopsy examination

	Number of patients
Total number of patients	77
SLN identified	72 (93.5%)
SLN positive	24 (33.3%)
SLN was only positive lymph node	11 (45.8%)
SLN identification method	
Radiocolloid and blue dye	53 (73.6%)
Radiocolloid only	11 (14.3%)
Blue dye only	8 (11.1%)

Table 3
Comparison of lymph node status of SLNs and non-SLNs

SLN status	Non-SLN status	
	Positive	Negative
Positive	13	11
Negative	3	45

False-negative rate = 11.1%.

11 of these patients (45.8%), the SLN was the only positive node. SLNs were identified by both radiocolloid and blue dye in 53 patients (73.6%), by radiocolloid alone in 11 patients (14.3%), and by blue dye alone in 8 patients (11.1%).

The pathologic status of the SLNs and non SLNs is shown in Table 3.

The SLNs accurately predicted the axillary status in 69 of 72 patients (95.8%). Three patients had a false-negative SLN biopsy examination result, resulting in a false-negative rate of 11.1% (3 of 27). Forty-five patients had pathologically negative SLNs and non-SLNs.

The pathologic status of the SLNs and non-SLNs were analyzed according to tumor classifications before NAC, clinical lymph node status before NAC, and response of the tumor after NAC, respectively.

In T2 tumors before NAC, the SLN identification rate was 94% (47 of 50), and 2 patients had a false-negative SLN biopsy examination result, resulting in a false-negative rate of 14.3%. In T3 and T4 tumors, results were 92.6% (25 of 27) and 7.7% (2 of 27), respectively (Table 4). For the results of SLN biopsy examination, there was no significant difference between T2 and T3/T4 tumors before NAC.

In the patients with clinically negative lymph nodes (N0) before NAC, the SLN identification rate was 97.6% (41 of 42), and 1 patient had a false-negative SLN biopsy examination result, resulting in a false-negative rate of 10%. In the patients with clinically positive lymph nodes (N1/N2), the results were 88.6% (31 of 35) and 11.2% (4 of 35), respectively (Table 5). The SLN identification rate tended to be higher, although not statistically significantly, among patients who had clinically negative lymph nodes before NAC compared with patients who had positive axillary lymph nodes before NAC.

Table 4
Comparison of lymph node status of SLNs and non-SLNs among tumor classifications before NAC

SLN status	Non-SLN status			
	T2 (n = 50)		T3/T4 (n = 27)	
	Positive	Negative	Positive	Negative
Positive	6	6	7	5
Negative	2	33	1	12
Total number of SLNs identified	47 (94%)		25 (92.6%)	
False-negative rate	14.3%		7.7%	

Table 5
Comparison of lymph node status of SLNs and non-SLNs among nodal status before NAC

SLN status	Non-SLN status			
	N0 (n = 42)		N1/N2 (n = 35)	
	Positive	Negative	Positive	Negative
Positive	3	6	10	5
Negative	1	31	2	14
Total number of SLNs identified	41 (97.6%)		31 (88.6%)	
False-negative rate	10%		11.2%	

For patients with complete tumor response after NAC, the SLN identification rate was 92.0% (37 of 41), with 1 patient having a false-negative SLN biopsy examination result, resulting in a false-negative rate of 12.5%. For patients with a partial tumor response and stable disease, the results were 97.2% (35 of 36) and 10.5% (1 of 36), respectively (Table 6). The SLN identification rate tended to be lower, although not statistically significantly, among patients with complete tumor response after NAC, compared with partial tumor response and patients with stable disease after NAC.

There was no significant difference in the false-negative rate according to tumor classifications before NAC, clinical lymph node status before NAC, and response of the tumor after NAC.

Comments

ALND is the surgical standard for treatment of the axilla in breast cancer patients. The rationales for ALND are exact staging and prognosis, regional control of the axilla, and the possibility of improved survival. The extent of axillary lymph node involvement is one of the most important independent prognostic factors for recurrence and survival. The SLN biopsy procedure is an accurate minimally invasive method for axillary staging in early breast cancers. In many clinics the SLN biopsy examination is replacing standard ALND because of minimal morbidity. However, with the increasing size of tumors, lymphatic mapping becomes

Table 6
Comparison of lymph node status of SLNs and non-SLNs among clinical response after NAC

SLN status	Non-SLN status			
	CR (n = 41)		PR/SD (n = 36)	
	Positive	Negative	Positive	Negative
Positive	3	4	10	7
Negative	1	29	2	16
Total number of SLNs identified	37 (90.2%)		35 (97.2%)	
False-negative rate	12.5%		10.5%	

Table 7
Studies of SLN biopsy procedures after NAC

	Number of patients	Stage	Tumor size, cm	Number (%) of successful SLN biopsy procedures	False negative (%)
Breslin et al [6], 2000	51	II or III	5.0	43 (84.3)	3 (12)
Miller et al [7], 2002	35	T1-3N0	3.5	30 (86.0)	0 (0)
Stearns et al [8], 2000	34	T3-4, any N	5.0	29 (85.0)	3 (14)
Haid et al [9], 2001	33	T1-3, any N	3.3	29 (88.0)	0 (0)
Julian et al [11], 2002	31	I or II	NS	29 (93.5)	0 (0)
Tafra et al [12], 2001	29	Any T, N0	NS	27 (93.0)	0 (0)
Nason et al [13], 2000	15	T2-4, N0	NS	13 (87.0)	3 (33)
Shimazu et al [14], 2004	47	II or III	4.5	44 (93.6)	4 (12)
Current study	77	T2-4, any N	4.8	72 (93.5)	3 (11)

NS = not specified.

less accurate [15,16]. NAC can reduce tumor size and significantly increase the ability to perform breast-conserving therapy [17,18]. After NAC, axillary downstaging is affected similarly. NAC with anthracycline/cyclophosphamide-containing regimens has been shown to neutralize involved axillary nodes in about 30% of patients [17]. The addition of taxanes to anthracycline/cyclophosphamide-containing regimens has increased the conversion rate to around 40% [19,20]. With the increasing number of patients receiving NAC, the question arises of whether the SLN biopsy examination is an option for these patients. We summarized the studies concerning SLN biopsy examination after NAC in Table 7, but they are inconclusive [6–14]. Breslin et al [6] reported a study of 51 patients who underwent an SLN biopsy examination after NAC and concluded that an SLN biopsy examination is accurate after NAC. They had an identification rate of 84.3% and a false-negative rate of 12.0%. Nason et al [13] reported on a smaller number of patients who received NAC. Their identification rate was 87.0% and their false-negative rate was 33.3%, concluding that the SLN biopsy examination resulted in an unacceptably high false-positive rate. We have to understand that in most of these small series, even 1 or 2 patients with a false-negative SLN node can sway the conclusions in a different direction. We report a study of 77 patients who received NAC, and had an identification rate of 93.5% and a false-negative rate of 11.1%. We conclude in our study that an SLN biopsy examination after NAC is accurate even for large tumors and positive axillary nodal status before NAC without inflammatory breast cancer.

It has been speculated that among patients who have their axillary lymph node status downstaged by NAC, tumors also typically respond to NAC and shrink, so that damage to and alteration of the lymphatic flow from tumor tissues to the axillary basin are more likely to occur. This may cause an increase in the false-negative rate for SLN biopsy examination and a decreasing identification rate for SLN biopsy examination. Our hypothesis is that the lymphatic flow around the skin lesion is rich and less influenced by the effect of chemotherapy and tumor size than that in the parenchyma around the tumor. Our results were not significantly influenced by tumor size, tumor response, or nodal status before NAC.

In conclusion, the results of our study suggest that an SLN biopsy procedure after NAC using intradermal injection of radiocolloid is feasible and can predict axillary lymph node status with high accuracy for patients with clinically negative lymph node status after NAC. This procedure could make patients who have had their axillary lymph node status downstaged from positive to negative and patients with large tumors appropriate candidates for an SLN biopsy examination.

Further studies involving a larger number of patients will be required to establish fully the feasibility and accuracy of the SLN biopsy procedure for patients with breast cancer who have been treated with NAC.

References

[1] Cody HS 3rd. Clinical aspects of sentinel node biopsy. Breast Cancer Res 2001;3:104–8.
[2] Cody HS, Borgen PI. State-of-the-art approaches to sentinel node biopsy for breast cancer: study design, patient selection, technique and quality control at Memorial Sloan-Kettering Cancer Center. Surg Oncol 1999;8:85–91.
[3] Krag D, Weaver D, Ashikaga T, et al. The sentinel node in breast cancer—a multicenter validation study. N Engl J Med 1998;339: 941–6.
[4] Anderson BO. Sentinel lymphadenectomy in breast cancer: an update on NCCN Clinical Practice Guidelines. J Natl Compr Cancer Network 2003;1(Suppl 1):S64–70.
[5] Reintgen D, Giuliano R, Cox C. Lymphatic mapping and sentinel lymph node biopsy for breast cancer. Cancer J 2002;8(Suppl 1):S15–21.
[6] Breslin TM, Cohen L, Sahin A, et al. Sentinel lymph node biopsy in accurate after neoadjuvant chemotherapy for breast cancer. J Clin Oncol 2000;18:3480–6.
[7] Miller AR, Thompson VE, Yeh IT, et al. Analysis of sentinel lymph node mapping with immediate pathologic review in patients receiving preoperative chemotherapy for breast carcinoma. Ann Surg Oncol 2002;9:243–7.
[8] Stearns V, Ewing CA, Slake R, et al. Sentinel lymphadenectomy after neoadjuvant chemotherapy for breast cancer may reliable represent the axilla except for inflammatory breast cancer. Ann Surg Oncol 2000;9:235–42.

[9] Haid A, Tausch C, Lang A, et al. Is sentinel lymph node biopsy reliable and indicated after preoperative chemotherapy in patients with breast cancer? Cancer 2001;92:1080–4.
[10] Julian TB, Patel N, Dusi D, et al. Sentinel node biopsy after neoadjuvant chemotherapy for breast cancer. Am J Surg 2001;182:407–10.
[11] Julian TB, Dusi D, Wolmark N. Sentinel node biopsy after neoadjuvant chemotherapy for breast cancer. Am J Surg 2002;184:315–7.
[12] Tafra L, Verbanac KM, Lannin DR. Preoperative chemotherapy and sentinel lymphadenectomy for breast cancer. Am J Surg 2001;182:312–5.
[13] Nason KS, Anderson BO, Byrd DR, et al. Increased false negative sentinel node biopsy rates after preoperative chemotherapy for invasive breast carcinoma. Cancer 2000;89:2187–94.
[14] Shimazu K, Tamaki Y, Taguchi T, et al. Sentinel lymph node biopsy using periareolar injection of radiocolloid for patients with neoadjuvant chemotherapy-treated breast carcinoma. Cancer 2004;100:2555–61.
[15] Bedrosian I, Reynolds C, Mick R, et al. Accuracy of sentinel lymph node biopsy in patients with large primary breast tumors. Cancer 2000;88:2540–5.
[16] O'Hea BJ, Hill AD, El-Shirbiny AM, et al. Sentinel lymph node biopsy in breast cancer: initial experience at Memorial Sloan-Kettering Cancer Center. J Am Coll Surg 1998;186:423–7.
[17] Fisher B, Brown A, Mamounas E, et al. Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from the National Surgical Adjuvant Breast and Bowel Project B-18. J Clin Oncol 1997;15:2483–93.
[18] Smith IC, Heys SD, Hutcheon AW, et al. Neoadjuvant chemotherapy in breast cancer: significantly enhanced response with docetaxel. J Clin Oncol 2002;20:1456–66.
[19] Mamounaus E, Brown A, Smith R, et al. Accuracy of sentinel node biopsy after neoadjuvant chemotherapy in breast cancer: update results from NSABP B-27. Proc Am Soc Clin Oncol 2002;21:36a.
[20] Gianni L, Baselga H, Eiermann W, et al. First report of European Cooperative Trial in operable breast cancer (ECTO): effect of primary systemic therapy (PST) on local-regional disease. Proc Am Soc Clin Oncol 2002;21:34a.

Breast Cancer
Vol. 13 No. 1 January 2006

Case Report

A Case of Mucinous Carcinoma of the Breast that Demonstrated a Good Pathological Response to Neoadjuvant Chemotherapy Despite a Poor Clinical Response

Junpei Yamaguchi, Sadako Akashi-Tanaka, Takashi Fukutomi, Takayuki Kinosita, Eriko Iwamoto, and Miyuki Takasugi

Breast Surgery Division, National Cancer Center Hospital, Japan.

A 30-year-old woman presented with a right breast tumor. Mucinous carcinoma was diagnosed by core needle biopsy (T2: 5 cm N1 M0). Despite receiving a neoadjuvant anthracycline and taxane regimen, the patient demonstrated no clinical response (NC). Based on the patient's strong preference, we performed breast-conserving surgery. On histological examination, we observed widespread mucus and a few viable malignant cells, a Grade 2 therapeutic response. Neither optimal management procedures nor guidelines for chemotherapy for primary mucinous carcinoma of the breast have been established. It is a reasonable assumption, however, that discordance between the clinical response and therapeutic response to neoadjuvant chemotherapy may occur in cases of mucinous carcinoma.

Breast Cancer 13:100-103, 2006.

Key words: Breast cancer, Mucinous carcinoma, Therapeutic response, Neoadjuvant chemotherapy

Neoadjuvant chemotherapy results in significant regression of primary breast carcinomas, thus allowing breast-conserving surgery. While a variety of imaging modalities are useful to estimate the extent of residual tumor[1,2], chemotherapy-induced fibrosis, tumor necrosis, and remaining fibrocystic changes make it difficult to evaluate the residual tumor load accurately. As far as we know, no reports have evaluated the responses to chemotherapy and neoadjuvant chemotherapy of mucinous carcinoma of the breast. In this report, we describe a case of breast mucinous carcinoma that demonstrated a pathological Grade 2 response, according to the histopathological response criteria of the Japanese Breast Cancer Society[3], despite a poor clinical response to neoadjuvant chemotherapy.

Case Report

A 30-year-old premenopausal woman was referred to our hospital with a lump in her breast. Physical examination revealed a hard elastic mass measuring 5 × 4.5 cm in diameter located in the upper outer quadrant of her right breast. An enlarged lymph node was also palpable in the right axilla. Mammography (MMG) displayed a well-circumscribed and high-density tumor shadow with microcalcifications (Fig 1A). The tumor measured approximately 5 cm in diameter by MMG. Ultrasonography (US) revealed an irregularly shaped hypoechoic lesion in the right breast, measuring over 5 cm in diameter (Fig 1B). The swollen lymph node was 1.5 cm in diameter, which was highly suggestive of lymph node metastasis. Serum levels of multiple tumor markers were normal; CEA levels were 2.0 ng/ml (normal: <5.0), CA15-3 was 6 U/ml (nl: <28), and ST439 was <1.0 U/ml (nl: <7.0). A core needle biopsy revealed mucinous carcinoma. Immunohistochemical analysis revealed no reactivity for either Estrogen receptor (ER) or Progesterone receptor (PgR). We did not observe immunoreactivity for p-53 or c-erbB-2 overexpression in this tumor.

The patient received neoadjuvant chemotherapy consisting of four cycles of 5FU (500 mg/m^2), epirubicin (100 mg/m^2), and cyclophosphamide (500 mg/m^2) every three weeks, followed by 12 cycles of paclitaxel (80 mg/m^2) weekly. The che-

Reprint requests to Junpei Yamaguchi, Breast Surgery Division, National Cancer Center Hospital, 5-1-1 Tukiji, Chuo-ku, Tokyo 104-0045, Japan.

Received January 7, 2005; accepted July 27, 2005

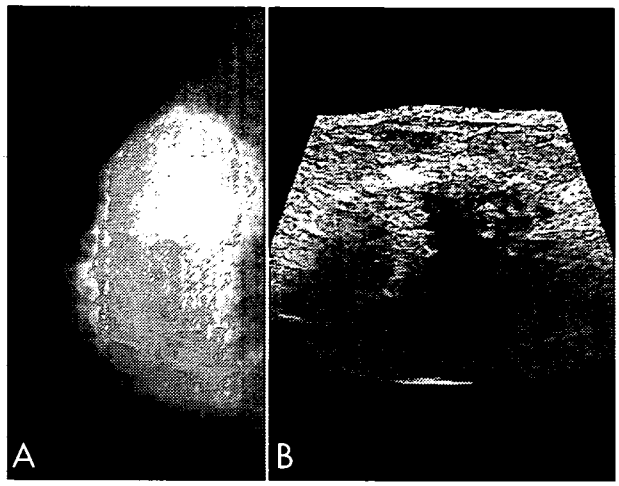


Fig 1. Mammography (MMG) showed a well-circumscribed, high-density tumor shadow with microcalcifications (A), while ultrasonography (US) revealed an irregularly shaped hypoechoic lesion in the right breast, measuring over 5 cm in diameter (B).

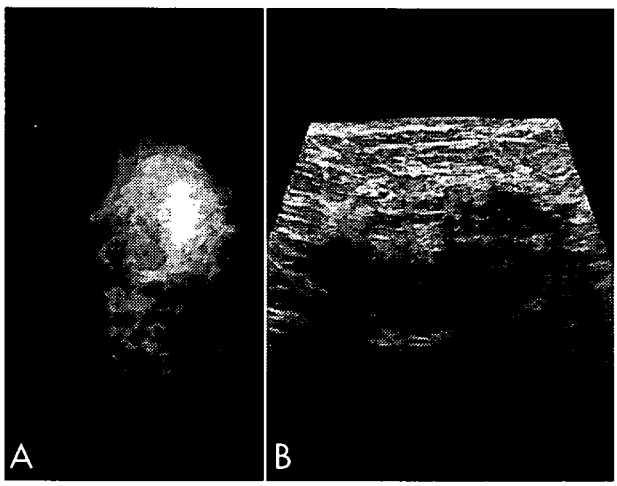


Fig 2. Additional imaging modalities (MMG (A) and US (B)) also revealed a tumor with similar size and features to that observed prior to the chemotherapy.

motherapeutic course was completed and the toxicities were tolerable. After the termination of chemotherapy, however, the tumor size remained unchanged. The tumor measured 4.5 × 4 cm in diameter by palpation, and the axillary lymph node was still palpable. The imaging examinations (MMG and US) also revealed a tumor of the same size with similar features as those seen prior to chemotherapy (Fig 2). Contrast-enhanced computed tomography (CE-CT) of the breast revealed an irregularly shaped faintly enhanced tumor shadow approximately 5 cm in diameter (Fig 3). Considering these features, we evaluated the clinical

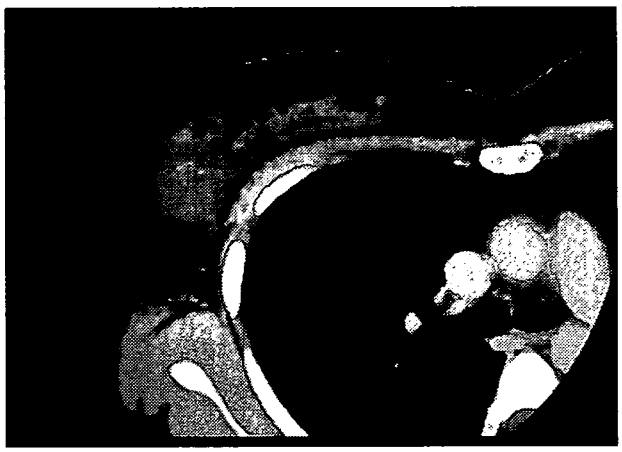


Fig 3. Contrast-enhanced computed tomography (CE-CT) of the breast revealed an irregularly shaped tumor shadow, which faintly enhanced and measured about 5 cm in diameter.

response to chemotherapy as no change (NC).

According to the patient's preference, we performed a wide resection of the tumor in the right breast with a level II lymph node dissection (Bp+Ax). The cut margin of the specimen was negative (free margin: 2 cm). Histologically, the tumor exhibited a pure infiltrating mucinous carcinoma, with no infiltrating ductal carcinoma component. The pathological tumor size was 5.0 cm in diameter and histological cut margin was also negative. Despite widespread mucus in the breast tumor, we recognized only a few viable malignant cells. The majority of the remaining tumor cells were necrotic (Fig 4A, 4B). According to the histopathological response criteria of the Japanese Breast Cancer Society, the pathological assessment of the therapeutic response was Grade 2[3]. We also recognized two swollen lymph nodes filled with mucus, but devoid of malignant cells.

Postoperatively, she received radiotherapy. She remains disease-free five months after the operation.

Discussion

Neoadjuvant chemotherapy has become standard therapy for patients with locally advanced or large operable breast cancers. This procedure makes breast-conserving surgery possible. In this report, we present a case of mucinous carcinoma, demonstrating a Grade 2 pathological response to neoadjuvant chemotherapy, despite no clinical response.

The reported incidence of mucinous carcino-

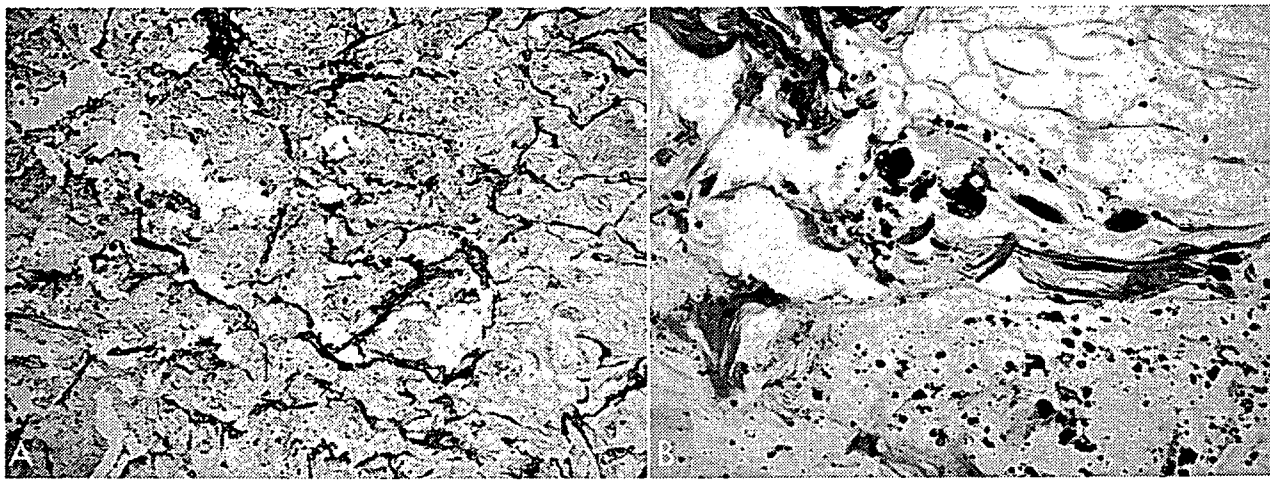


Fig 4. A: Despite widespread mucus in the primary breast tumor, microscopic analysis could recognize only a few viable malignant cells. The majority of these tumor cells were necrotic (hematoxylin and eosin [H&E] stain, original magnification, ×40), B: Viable malignant cells (H&E stain, original magnification, ×200).

ma has varied from 1% to 6% of all breast malignancies. Mucinous carcinoma has a better prognosis than infiltrating ductal carcinomas[4-6]. Komenaka *et al.* reported that the number of involved axillary lymph nodes was the only significant predictor of death from disease; the size of the lesion was not a significant prognostic factor in mucinous carcinoma, because the mucin comprised the majority of the tumor volume[5]. Pure mucinous carcinoma of the breast is suitable for breast-conserving therapy, even for large tumors of up to 5 cm in diameter, because these tumors have a low incidence of extensive intraductal spreading[6]. There have not been, however, any reports detailing the typical responses to chemotherapy or neoadjuvant chemotherapy for mucinous carcinoma of the breast. Mucinous carcinomas tend to be identified early, when the tumors are small in size and neoadjuvant chemotherapy is often unnecessary. According to multiple studies, tumors with aggressive biological markers, such as high histological grade, overexpression of HER-2, reactivity for p-53, and negative hormone receptor status, exhibited better pathological responses[7-9]. In mucinous carcinomas, estrogen and progesterone receptor positivity have been reported in approximately 60-90% of the tumors, while HER2/neu oncoprotein overexpression and p53 protein accumulation are not normally seen. These biological features suggest that mucinous carcinomas may not respond well to neoadjuvant chemotherapy. Fortunately, this estimation did not fit this case; the negative hormone receptor status of this tumor may be associated with a good response to neoadjuvant chemotherapy.

Categorization of the clinical response to chemotherapy depends on an accurate measurement of residual tumor size, but is complicated by variable histopathologic changes that can occur within the tumor bed. The remaining tumorous lesions may be related to chemotherapy-induced fibrosis, tumor necrosis, or fibrocystic changes. These secondary processes can result in clinical and macroscopic overestimation of the residual tumor size[10, 11]. Rajan *et al.* reported that chemotherapy in some tumors can dramatically reduce cellularity, but only minimally affects the overall tumor size[12]. In this case, chemotherapy was profoundly effective against the tumor cells themselves, but the large amounts of extacellular mucus were not sensitive to chemotherapy; thus, the remaining mucus made up a significant portion of the residual tumor. This phenomenon resulted in a discordance between the residual tumor size and the effectiveness of chemotherapy. Meanwhile, we observed five patients with pure type mucinous carcinoma that received neoadjuvant chemotherapy at our hospital (Table 1). The pathological assessments of the therapeutic responses of these tumors were Grade 1a (two cases), Grade 1b (two cases) and Grade 2 (this case) according to the percentage of necrotic malignant cells. Interestingly, in the two Grade 1b cases, approximately two-thirds of the tumor cells were necrotic, but large amounts of mucus remained. Due to the small number and varied responses, we could not

Table 1. Pure Type Mucinous Carcinoma of the Breast Receiving Neoadjuvant Chemotherapy

case	age	tumor size (cm)	regimen	tumor size (cm) postchemotherapy	clinical response	pathological response	ER	PgR
1	31	7	AT	4.9	PR	1a	−	−
2	39	3.5	AT	3.5	NC	1a	++	+
3	61	6.5	AC→PTX	3.5	PR	1b	++	+/−
4	53	1.3	AT→PTX	0.5	PR	1b	+	+/−
this case	30	5	CEF→PTX	5	NC	2	−	−

AT: adriamycin + docetaxel, AC: adriamycin + cyclophosphamide, PTX: paclitaxel, CEF: cyclophosphamide + epirubicin + 5FU

recognize a general trend in the responses to chemotherapy. These characteristic findings upon pathological examination, however, indicated that mucinous carcinomas may not be reduced in size even when the tumor cells are sensitive to chemotherapy.

In conclusion, discordance between residual tumor size and the effectiveness of chemotherapy may be observed in mucinous carcinoma. More detailed studies are required to establish the indications for chemotherapy and to evaluate therapeutic responses in patients with mucinous carcinoma.

References

1) Akashi-Tanaka S, Fukutomi T, Watanabe T, *et al*: Accuracy of contrast-enhanced computed tomography in the prediction of residual breast cancer after neoadjuvant chemotherapy. *Int J Cancer (Radiat Oncol Invest)* 96:66-73, 2001.
2) Akashi-Tanaka S, Fukuotmi T, Sato N, *et al*: The use of contrast-enhanced computed tomography before neoadjuvant chemotherapy to identify patients likely to be treated safely with breast-conserving surgery. *Ann Surg* 239:238-243, 2004.
3) The Japanese Breast Cancer Society: General rules for clinical and pathological recording of breast cancer, 14th ed. Kanehara, Tokyo, 2000.
4) Andre S, Cunha F, Bernardo M, *et al*: Mucinous carcinoma of the breast: a pathological study of 82 cases. *J Surg Oncol* 58:162-167, 1995.
5) Ian K. Komenaka, Mahmoud B. El-Tamer, *et al*: Pure mucinous carcinoma of the breast. *Am J Surg* 187:528-532, 2004.
6) Anan K, Mitsuyama S, Tanaka K, *et al*: Pathological features of mucinous carcinoma of the breast are favorable for breast-conserving therapy. *EJSO* 27:459-463, 2001.
7) Henry M. Kuerer, Lisa A. Newman, Terry L. Smith, *et al*: Clinical course of breast cancer patients with complete pathologic primary tumor and lymph node response to doxorubicin-based neoadjuvant chemotherapy. *J Clin Oncol* 17:460-469, 1999.
8) AH Honkoop, PJ van Diest, JS de Jong, *et al*: Prognostic role of clinical, pathological and biological characteristics in patients with locally advanced breast cancer. *British J Cancer* 77:621-626, 1998.
9) Frederique Penault-llorca, Anne Cayre, Florence Bouchet Mishellany, *et al*: Induction chemotherapy for breast carcinoma: predictive markers and relation with outcome. *Int J Oncol* 22:1319-1325, 2003.
10) Nakamura T, Fukutomi T, Tsuda H, *et al*: Changes in findings of mammography, ultrasonography and contrast-enhanced computed tomography of three histological complete responders with primary breast cancer before and after neoadjuvant chemotherapy: case reports. *Jpn J Clin Oncol* 30:453-457, 2000.
11) Edwin R. Fisher, Jiping Wang, John Bryant, *et al*: Pathobiology of preoparative chemotherapy. Findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) Protocol B-18. *Cancer* 95:681-694, 2002.
12) Radhika Rajan, Anna Poniecka, Terry L. Smith, *et al*: Change in tumor cellularity of breast carcinoma after neoadjuvant chemotherapy as a variable in the pathologic assessment of response. *Cancer* 100:1365-1373, 2004.

講座

術前化学療法後のセンチネルリンパ節生検

木下貴之*1　福富隆志*1　関　邦彦*2

Sentinel Lymph Node Biopsy for Breast Cancer Patients after Neoadjuvant Chemotherapy：Kinoshita T*1, Fukutomi T*2, Seki K*3 (*1,2Surgical Oncology Division, *3Department of Pathology, National Cancer Center)

Despite the increasing use of both sentinel node biopsy and neoadjuvant chemotherapy in patients with operable breast cancer, there is still limited information on the feasibility and accuracy of sentinel node biopsy following neoadjuvant chemotherapy. So, the feasibility and accuracy of sentinel lymph node (SLN) biopsy for breast cancer patients with clinically node negative after neoadjuvant chemotherapy (NAC) has been investigated under the administration of a radiocolloid imaging agent injected intradermally over a tumor. Also, conditions which may affect SLN biopsy detection and false-negative rates with respect to clinical tumor response and clinical nodal status before NAC were also analyzed.

Our results show that SLN identification rate and false-negative rate after NAC are similar to those in nonneoadjuvant studies.

Key words：Breast cancer patients, After neoadjuvant chemotherapy, Sentinel node biopsy
Jpn J Breast Cancer **21**(2)：135～139, 2006

はじめに

近年，センチネルリンパ節生検による腋窩郭清の省略と術前化学療法の併用により乳癌の外科治療は急速に縮小化の方向に進んでいる．センチネルリンパ節生検は，1990年代に始まり，従来の色素法にRIを用いたガンマプローブ法を組み合わせるなどの技術的改良と外科医自身の学習効果により，その成績も90%を超える同定率と5～10%の偽陰性率の達成が可能になってきている[1]．海外における69の施設と10,000人以上の患者を対象とした早期乳癌に対するセンチネルリンパ節生検のメタアナリシスの結果は，全体の同定率が90%以上で偽陰性率も8.4%と報告されている[2]．センチネルリンパ節生検の結果，腋窩郭清の省略が可能になった患者は，腋窩郭清を施行された患者と比較して術後合併症の頻度が低く，患手のむくみ，痺れ，運動障害などが軽度でQOLもより良好であると考えられる[1]．海外におけるセンチネルリンパ節生検の比較試験の長期的な成績が待たれるが，本邦においても多くの施設が既にセンチネルリンパ節生検の安全性試験を終了し実地医療へと移行しているものと考えられる．

一方，術前化学療法の導入により多くの症例でダウンステージ効果により乳房温存療法が可能になってきた．術前化学療法は従来，病期IIIB以上のいわゆる局所進行癌を対象に非切除例を切除可能にする目的で実施されてきたが，近年は病期IIAからIIIAの症例も術前化学療法の対象とし，原発巣が巣縮小した結果，多くの症例で乳房温存療法が可能となっている．これらの効果は，原発巣ばかりではなく当然，腋窩リンパ節転移巣にも確認されている．アンスラサイクリン系を含む術前化学療法では，腋窩リンパ節転移を約30%減じ[3]，さらにタキサン系を加えたレジメンでは約40%減ずると報告されている[4,5]．当院

*1 国立がんセンター中央病院外科
*2 国立がんセンター中央病院病理診断部

では1998年から2005年まで約360例の乳癌症例に術前化学療法を実施してきた．術前化学療法の原発巣における効果は，約85%以上の症例がPR以上であった．約25%の症例は原発巣がCRとなったが，これらの症例の腋窩リンパ節転移陽性率は25%で，早期乳癌のそれとほぼ同程度まで低下していることが確認された．このような術前化学療法が著効した症例に対して早期乳癌と同様にセンチネルリンパ節生検を実施し，腋窩郭清を省略することが可能かどうかを明らかにすることは非常に重要な課題である．

1．術前化学療法後のセンチネルリンパ節生検における問題点

術前化学療法後のセンチネルリンパ節生検に関してはいまだ十分なエビデンスは得られていない．これまでの報告例はいずれも単一施設で少数例の結果であり大規模な臨床試験は行われていない．早期乳癌症例に対するセンチネルリンパ節生検と比較すると，術前化学療法後の症例の問題点は，①腫瘍径の大きな症例が対象になる，②腋窩リンパ節転移の存在する，または存在した症例がより多く含まれる，③術前化学療法が腫瘍－リンパ管－リンパ節の流れに影響を与える可能性がある，④術前化学療法は転移陽性であったセンチネルリンパ節とノンセンチネルリンパ節に同程度の効果があるのか？⑤術前化学療法後のn0の意義がまだ明らかになっていない，などが挙げられる．これらの要因が術前化学療法のセンチネルリンパ節生検の妥当性を検証するうえで問題点となってきた．

1）海外での成績

術前化学療法後のセンチネルリンパ節生検のこれまで報告されてきた単一施設の成績を**表1**にまとめた[6~13]．症例数は15例から51例といずれも少数例での報告となっている．腫瘍径は平均で3.3 cmから5.5cmで，T1からT4まで対象とし，また，リンパ節転移が認められる症例も含めた試験も報告されている．これらのセンチネルリンパ節の同定率は84%から93%程度で，早期乳癌の成績よりやや低い程度である．偽陰性率は，0%から33%とばらつきを認める．これら7施設の報告をまとめると全体としての同定率は88.7%で，偽陰性率は5.3%である．ただし，偽陰性率に関してはNasonらの15例での33%という報告と少数例を対象にした0%という報告を除けば10%～15%程度という成績が臨床的にも妥当なのではないかと推測する．

術前化学療法後のセンチネルリンパ節生検のこれまで報告されてきた多施設の成績を**表2**にまとめた[14~17]．MamounasらはNational Surgical Adjuvant Breast and Bowel Project randomized trial (NSABP B-27) のAC4サイクルにdocetaxelを加えた術前化学療法後にセンチネルリンパ節生検が試みられた428例の成績を報告している[14]．試験が多施設にわたるためセンチネルリンパ節生検手技は，まちまちであるが全体としての同定率は85%，偽陰性率は11%という結果である．その他の3つの多施設からの報告も同定率が90%前後，偽陰性率が10%前後と早期乳癌に対するセンチネルリンパ節生検の成績と遜色のない結果が報告されている．

また，これらの結果からわかることは，術前化学療法後にセンチネルリンパ節生検を行う際には，色

表1　術前化学療法後センチネルリンパ節生検－単施設の成績－

	症例数	病期	平均腫瘍径(cm)	同定率(%)	偽陰性率(%)
Breslin et al.,2000[6]	51	II or III	5.0	43(84.3)	3(12)
Miller et al.,2002[7]	35	T1-3N0	3.5	30(86.0)	0(0)
Stearns et al.,2000[8]	34	T3-4, any N	5.0	29(85.0)	3(14)
Haid et al.,2001[9]	33	T1-3, any N	3.3	29(88.0)	0(0)
Julian et al.,2002[10]	31	I or II	NS	29(93.5)	0(0)
Tafra et al.,2001[11]	29	Any T, N0	NS	27(93.0)	0(0)
Nason et al.,2000[12]	15	T2-4, N0	NS	13(87.0)	3(33)
Shimazu et al.,2004[13]	47	II or III	4.5	44(93.6)	4(12)
Kinoshita et al., 2005	88	T2-4, any N	4.9	81(92.0)	3(9)

表2　術前化学療法後センチネルリンパ節生検－多施設の成績－

[illegible]	[illegible]	[illegible]	[illegible](%)	[illegible](%)
Mamounas et al[14]	428	Blue dye	78	14
(NSABP B-27)		Radiocolloid	89	5
		Combination	88	9
		All techniques	85	11
Krag et al[15]	443	Radiocolloid	93	11
Tafra et al[16]	529	Combination	87	13
McMaster et al[17]	806	Blue dye or Radiocolloid	86	12
		Combination	90	6
		All Techniques	88	7

表3　患者背景

[illegible]	[illegible]
平均年齢(歳)	50.2 (27-77)
平均腫瘍径 (cm)*	4.91 (2.7-12)
T分類*	
T2	54 (61%)
T3	28 (32%)
T4	6 (7%)
N分類*	
N0	46 (52%)
N1	34 (39%)
N2	8 (9%)
組織型	
浸潤性乳管癌	86 (98%)
浸潤性小葉癌	2 (2%)
術前化学療法	
FEC plus paclitaxel	85 (97%)
paclitaxel alone	3 (3%)
臨床的腫瘍効果	
CR	45 (51%)
PR	35 (40%)
NC	8 (9%)
病理組織学的腫瘍効果	
pCR	34 (39%)
pINV	54 (61%)
リンパ節転移	
陰性	38 (43%)
陽性	50 (57%)

*化学療法前
pCR＝pathological complete response；pINV＝pathological invasive

素法単独より色素法にRI法を併用した方が成績がよいということである．

2) 国立がんセンターの成績

当院では，早期乳癌に対するセンチネルリンパ節生検のfeasibility studyを終了後，2003年7月より術前化学療法後の乳癌症例に対するセンチネルリンパ節生検のfeasibility studyを開始し，その成績を報告してきた．本試験は単一の外科医，手技により実施された．

腫瘍径3cm以上あるいは腋窩リンパ節転移を認める乳癌症例を対象に術前化学療法として，①FEC/ACを4サイクル，②weekly paclitaxelを12サイクルを組み合わせたものを原則とし，高齢者にのみ②だ

表4　国立がんセンターにおけるセンチネルリンパ節生検の成績

	非センチネルリンパ節の転移	
センチネルリンパ節の転移	陽性	陰性
陽性	16	14
陰性	3	48

False negative rate, 9.1%; overall accuracy, 96.3%; negative predictive value, 94.1%; positive predictive value, 100%

け実施した．術前化学療法後に原発巣がPR以上の効果を示し，かつ，治療後腋窩リンパ節転移が陰性であった88例をセンチネルリンパ節生検の対象とした．これらの平均腫瘍径は4.9cm（2.5cm～12.0cm）で，T4が6例，治療前に明らかにリンパ節転移を認めた42例も対象となっている（**表3**）．センチネルリンパ節生検は，色素－RI法を用いたものが80例で，色素法単独が8例となっている．結果として，センチネルリンパ節が同定できた症例は80例で，同定率は92%となる．これらの症例のセンチネルリンパ節とノンセンチネルリンパ節の転移の有無をまとめたものを**表4**に示す．センチネルリンパ節に転移を認めず，ノンセンチネルリンパ節に転移を認めたものは3例で偽陰性率は9%であり，全体として96%の症例においてセンチネルリンパ節が腋窩リンパ節全体の状況を正確に反映していることが証明された．臨床的諸因子とセンチネルリンパ節の同定率との関連を検討したが，治療前のリンパ節転移の有無，臨床的治療効果，病理組織学的治療効果は関連せず，唯一，T4d（炎症性乳癌）症例のみがセンチネルリンパ節の同定を困難にしていることが明らかとなった．一方，センチネルリンパ節が同定できた症例中，偽陰性になった症例は3例のみであったため，術前化学療法も含めてこれらに影響を与える因子は明らかではなかった．

まとめ

当院での術前化学療法後センチネルリンパ節生検の結果から，炎症性乳癌以外の術前化学療法が著効した症例において，センチネルリンパ節生検は十分に安全に実施できると結論づけられた．同定率は92%，偽陰性率は9%で，早期乳癌における成績と遜色のないものとなった．海外における最近の報告や多施設からの報告は，当院の結果を支持するものである．一方，2005年度にJournal of Clinical Oncology（JCO）に発表されたAmerican Society of Clinical Oncology（ASCO）のガイドラインでは，Preoperative systemic therapy後のセンチネルリンパ節生検に関して，①技術的には安全に実施することはできる，②Preoperative systemic therapy後のn0の意義が明らかでない，③これらの症例では，正確な腋窩リンパ節の転移状況の把握が治療方針を決める際に重要であること，④エビデンスが十分でない，ことより推奨されていない．正確な腋窩リンパ節の情報を得るという目的からするとセンチネルリンパ節生検をPreoperative systemic therapyの前に施行し，Preoperative systemic therapy後に実施する場合でもN0症例に限られるべきだと強調している[18]．

当院での成績から，強力で安定した化学療法の後，色素－RI法を用い熟練した手技のもとにセンチネルリンパ節生検は，安全に実施できることが確認された．術前化学療法が著効した乳癌症例では，腋窩リンパ節陽性率が25%程度になることから術前化学療法後にセンチネルリンパ節生検を実施することに意義があるものと考える．ただし，本対象が進行癌であるということを十分に認識し，腫瘍内科医，病理医，放射線診断医との連携のもとに，慎重に適応を決めて本手技を修練，実施することが望まれる．

文　献

1）Veronesi U, Pagenelli G, Viale G, et al：A randomized comparison of sentinel-node biopsy with routine axillary dissection in breast cancer. *N Engl J Med* **349**：546-553, 2003

2) Kim T, Agboola O, Lyman GH, et al : Lymphatic mapping and sentinel lymph node sampling in breast cancer : meta-analysis. *Proc Am Soc Clin Oncol* **21** : 36a, 2002
3) Fisher B, Brown A, Mamounas E, et al : Effect of preoperative chemotherapy of local-regional disease in women with operable breast cancer : findings from National Surgical Adjuvant Breast and Bowel Project B-18. *J Clin Oncol* **15** : 2483-2493, 1997
4) Mamounaus E, Brown A, Smith R, et al : Accuracy of sentinel node biopsy after neoadjuvant chemotherapy in breast cancer : update results from NSABP B-27. *Proc Am Soc Clin Oncol* **21** : 36a, 2002
5) Gianni L, Baselga H, Eiermann W, et al : First report of European Cooperative Trial in operable breast cancer (ECTO) : effect of primary systemic therapy (PST) onlocal-regional disease. *Proc Am Soc Clin Oncol* **21** : 34 a, 2002
6) Breslin TM, Cohen L, Sahin A, et al. Sentinel lymph node biopsy in accurate after neoadjuvant chemotherapy for breast cancer. *J Clin Oncol* **18** : 3480-3486, 2000
7) Miller AR, Thompson VE, Yeh IT, et al : Analysis of sentinel lymph node mapping with immediate pathologic review in patients receiving preoperative chemotherapy for breast carcinoma. *Ann Surg Oncol* **9** : 243-247, 2002
8) Stearns V, Ewing CA, Slake R, et al : Sentinel lymphadenectomy after neoadjuvant chemotherapy for breast cancer may reliable represent the axilla except for inflammatory breast cancer. *Ann Surg Oncol* **9** : 235-242, 2000
9) Haid A, Tausch C, Lang A, et al : Is sentinel lymph node biopsy reliable and indicated after preoperative chemotherapy in patients with breast cancer? *Cancer* **92** : 1080-1084, 2001
10) Julian TB, Dusi D, Wolmark N : Sentinel node biopsy after neoadjuvant chemotherapy for breast cancer. *Am J Surg* **184** : 315-317, 2002
11) Tafra L, Verbanac KM, Lannin DR : Preoperative chemotherapy and sentinel lymphadenectomy for breast cancer. *Am J Surg* **182** : 312-315, 2001
12) Nason KS, Anderson BO, Byrd DR, et al : Increased false negative sentinel node biopsy rates after preoperative chemotherapy for invasive breast carcinoma. *Cancer* **89** : 2187-2194, 2000
13) Shimazu K, Tamaki Y, Taguchi T, et al : Sentinel lymph node biopsy using periareolar injection of radiocolloid for patients with neoadjuvant chemotherapy-treated breast carcinoma. *Cancer* **100** : 2555-2561, 2004
14) Mamounas E, Brown A, Anderson S, et al : Sentinel node biopsy after neoadjuvant chemotherapy in breast cancer : Results from National Surgical Adjuvant Breast and Bowel Project Protocol B-27. *J Clin Oncol* **23** : 2694-2702, 2005
15) Krag D, Weavre D, Ashikaga T, et al : The sentinel node in breast cancer－A multicenter validation study. *N Engl J Med* **339** : 941-946, 1998
16) Tafra L, Lannin DR, Swason MS, et al : Multicenter trial of sentinel node biopsy for breast cancer using both technetium sulfur colloidal and isosulfan blue dye. *Ann Surg* **223** : 51-59, 2001
17) McMaster KM, Tuttle TM, Carison DJ, et al : Sentinel lymph node biopsy for breast cancer : A suitable alternative to routine axillary dissection in multi-institutional practice when optimal technique in used. *J Clin Oncol* **18** : 2560-2566, 2000
18) Lyman GH, Giuliano MR, Somerfield MR, et al : American Society of Clinical Oncology guideline recommendation for sentinel lymph node biopsy in early-stage breast cancer. *J Clin Oncol* **23** : 7703-7720, 2005

VOLUME 25 · NUMBER 4 · FEBRUARY 1 2007

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Dofequidar Fumarate (MS-209) in Combination With Cyclophosphamide, Doxorubicin, and Fluorouracil for Patients With Advanced or Recurrent Breast Cancer

Toshiaki Saeki, Tadashi Nomizu, Masakazu Toi, Yoshinori Ito, Shinzaburo Noguchi, Tadashi Kobayashi, Taro Asaga, Hironobu Minami, Naohito Yamamoto, Kenjiro Aogi, Tadashi Ikeda, Yasuo Ohashi, Wakao Sato, and Takashi Tsuruo

From the National Shikoku Cancer Center, Matsuyama; Hoshi General Hospital, Koriyama; Japanese Foundation for Cancer Research, Tokyo; School of Medicine, Osaka University, Osaka; Nihon Schering K.K., Osaka; Kanagawa Cancer Center, Yokohama; National Cancer Center Hospital East, Kashiwa; Chiba Cancer Center, Chiba; Tokyo Metropolitan Komagome Hospital; The Jikei University School of Medicine; School of Medicine, Keio University; Biostatistics/Epidemiology and Preventive Health Sciences, University of Tokyo; Institute of Molecular and Cellular Biosciences, University of Tokyo; and Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan.

Submitted July 17, 2006; accepted November 1, 2006; published online ahead of print at www.jco.org on December 18, 2006.

Supported by Schering AG, Berlin, Germany.

Presented in part at the 29th European Society for Medical Oncology Congress, Vienna, Austria, October 29–November 2, 2004, and the 27th San Antonio Breast Cancer Conference, San Antonio, TX, December 8-11, 2004.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Address reprint requests to Toshiaki Saeki, MD, PhD, Saitama Medical School Hospital, 38 Morohongo, Moroyama, Iruma-gun, Saitama 350-0495, Japan; e-mail: tsaeki@saitama-med.ac.jp.

0732-183X/07/2504-411/$20.00

DOI: 10.1200/JCO.2006.08.1646

A B S T R A C T

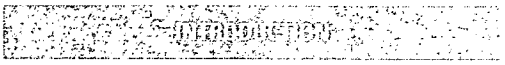
Purpose
To evaluate the efficacy and tolerability of dofequidar plus cyclophosphamide, doxorubicin, and fluorouracil (CAF) therapy in comparison with CAF alone, in patients with advanced or recurrent breast cancer. Dofequidar is a novel, orally active quinoline derivative that reverses multidrug resistance.

Patients and Methods
In this randomized, double-blind, placebo-controlled trial, patients were treated with six cycles of CAF therapy: 28 days/cycle, with doxorubicin (25 mg/m^2) and fluorouracil (500 mg/m^2) administered on days 1 and 8 and cyclophosphamide (100 mg orally [PO]) administered on day 1 through 14. Patients received dofequidar (900 mg PO) 30 minutes before each dose of doxorubicin. Primary end point was overall response rate (ORR; partial or complete response). In total, 221 patients were assessable.

Results
ORR was 42.6% for CAF compared with 53.1% for dofequidar + CAF, a 24.6% relative improvement and 10.5% absolute increase (P = .077). There was a trend for prolonged progression-free survival (PFS; median 241 days for CAF *v* 366 days for dofequidar + CAF; P = .145). In retrospectively defined subgroups, significant improvement in PFS in favor of dofequidar was observed in patients who were premenopausal, had no prior therapy, and were stage IV at diagnosis with an intact primary tumor. Except for neutropenia and leukopenia, there was no statistically significant excess of grade 3/4 adverse events compared with CAF. Treatment with dofequidar did not affect the plasma concentration of doxorubicin.

Conclusion
Dofequidar + CAF was well tolerated and is suggested to have efficacy in patients who had not received prior therapy.

J Clin Oncol 25:411-417. © 2007 by American Society of Clinical Oncology



Despite the advances in chemotherapeutic intervention, many cancers are either inherently resistant or develop resistance to chemotherapy.[1,2] Consequently, multidrug resistance (MDR) remains a major obstacle to the successful treatment of cancer.[1,3,4] One mechanism by which MDR operates is via the increased cellular efflux of cytotoxic compounds due to increased expression of membrane transport proteins such as P-glycoprotein (P-gp) and MDR-associated protein (MRP).[1,4,5] MDR affects many structurally and functionally unrelated agents including cytotoxic drugs that are hydrophobic, natural products, such as taxanes, vinca alkaloids, anthracyclines, epipodophyllotoxins, topotecan, dactinomycin, and mitomycin.[1,6,7] These represent some of the most commonly used chemotherapeutic agents.

In tumors with low levels of P-gp expression at baseline or diagnosis, P-gp expression increases after exposure to chemotherapy agents, thus leading to the development of MDR. In breast cancer patients who had received prior chemotherapy, P-gp expression has been shown to increase from 11% in untreated patients to 30% after chemotherapy.[8] Furthermore, compared with P-gp–negative tumors, a significant increase in resistance to paclitaxel and doxorubicin was reported in P-gp positive breast cancer tissue, irrespective of prior therapy.

The degree of P-gp expression also strongly correlated with the degree of drug resistance observed.[8]

Chemotherapy remains the treatment of choice for women with hormone receptor–negative and hormone-refractory breast cancer disease.[9-11] However, many tumors that are initially responsive to chemotherapy frequently relapse and develop resistance to the broad spectrum of cytotoxic drugs currently employed.[8,12,13] Consequently, MDR remains a major reason for treatment failure in patients with metastatic breast cancer and highlights the urgent need for MDR modifiers in breast cancer chemotherapy.

Since the discovery of verapamil as an MDR-reversing agent,[14] many compounds have been investigated as MDR inhibitors.[14-16] Dofequidar fumarate (Fig 1), is a novel, orally active, quinoline-derived inhibitor of MDR.[17] In preclinical studies, dofequidar reversed MDR in P-gp– and MRP-1–expressing cancer cells in vitro (1 to 3 μmol/L), as well as enhancing the antitumor effects of doxorubicin in MDR tumor–bearing mice.[17-19] A phase I trial in healthy volunteers showed dofequidar to be well tolerated (10 to 1,200 mg) with no dose-limiting toxicities and an effective plasma concentration was maintained for 8 hours at 900 mg (data on file, Schering AG, Berlin, Germany). In a phase II combination trial in patients with recurrent breast cancer, dofequidar potentiated the antitumor effects of CAF (cyclophosphamide, doxorubicin, and fluorouracil) therapy; patients who had not responded to treatment with three cycles of CAF responded to subsequent treatment with dofequidar plus CAF. The numbers of patients with an objective response were two of seven at 600 mg and two of six at 900 mg dofequidar, though dose escalation was stopped at 1,200 mg due to increased hematologic toxicity (data on file, Schering AG). On the basis of this result, this phase III study was conducted to compare the efficacy and safety of dofequidar plus CAF with placebo plus CAF in patients with advanced or recurrent breast cancer.

Study Design

This was a randomized, multicenter, double-blind, placebo-controlled trial conducted at 46 centers across Japan, comparing the efficacy and safety of dofequidar plus CAF with placebo plus CAF. Female patients (age 20 to 70 years) with advanced (stage IV at diagnosis with an intact primary tumor) or recurrent breast cancer were enrolled onto the study. Other inclusion criteria included a histologically defined, measurable or assessable primary lesion; two or fewer regimens of prior chemotherapy in both neo/adjuvant and metastatic settings, (excluding prior endocrine or single-agent fluorouracil therapy); 180 mg/m^2 anthracyclines (doxorubicin equivalent) or less previously; a performance status of 0 to 2; and adequate bone marrow, renal, hepatic and cardiac functions. Patients who progressed or had a recurrence in less than 6 months with anthracycline-containing chemotherapy, and those who had a history of major cardiac disease, uncontrolled hypertension, symptomatic brain metastasis, or simultaneous malignancy were excluded. The trial was approved by the institutional review board and was conducted in accordance with the Declaration of Helsinki (1996). All patients provided written informed consent before study entry.

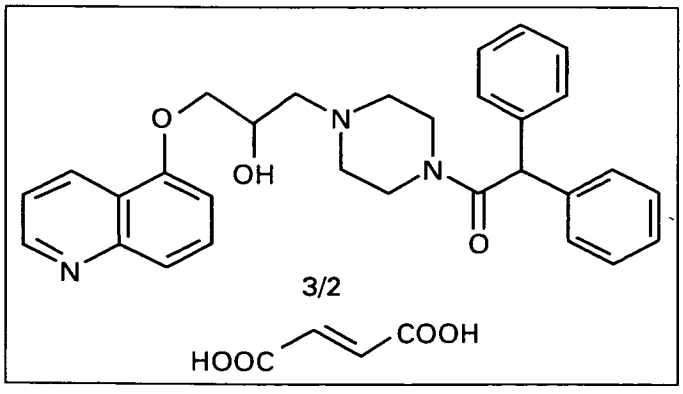


Fig 1. Structure of dofequidar (MS-209).

Dosing and Dose Modification for Toxicity

Patients were treated with six cycles of CAF therapy with dofequidar or placebo, and each treatment cycle lasted for 28 days; drugs were administered as follows: days 1 and 8, doxorubicin (25 mg/m^2) and fluorouracil (500 mg/m^2), each infused over 15 minutes; days 1 through 14, cyclophosphamide (100 mg orally [PO]); dofequidar (900 mg/d; 3 × 300 mg tablets) or placebo administered 30 minutes before each doxorubicin dose to ensure adequate blood concentration of dofequidar. The doses of doxorubicin and fluorouracil were reduced to 20 mg/m^2 and 400 mg/m^2, respectively, if any of the following criteria were met: grade 3 nonhematologic toxicity (except nausea and vomiting); grade 3 or worse neutropenia (< 1,000/mm^3) maintained for at least 5 days with an episode of fever of 38.5°C or higher; grade 3 or worse thrombocytopenia (< 50,000/mm^3); and grade 4 neutropenia (< 500/mm^3). The next cycle was postponed for 3 weeks unless the patient had a WBC count of at least 4,000/mm^3, or a neutrophil count of at least 2,000/mm^3 and a platelet count of at least 100,000/mm^3. Patients were followed up for 3 months after completion or discontinuation of treatment.

Treatment Assignment

Patients were randomly assigned to their treatment by the Trial Register Center. Treatment assignment was securely stored and coded until completion of the study. Investigators were also blinded to the assigned treatment. Patients were stratified by the number of prior chemotherapy regimens, including adjuvant chemotherapy, by a history of prior use of anthracyclines, and by the presence of liver metastases.

Efficacy

The primary study end point was the overall response rate (ORR) in the full analysis set (FAS; all patients who received treatment at least once and met all inclusion/exclusion criteria). Efficacy assessment by lesion and ORR assessment were made at each treatment cycle (every 4 weeks) and at treatment completion. Objective responses were assessed through blinded reading of radiographs by an independent expert panel. The secondary study end points included complete response rate (CR), time to treatment failure (TTF), time to progression (TTP), and progression-free survival (PFS).

Subgroup analyses were conducted to assess PFS within specific patient subpopulations, including premenopausal women, patients who had no prior therapy, and patients who had advanced primary breast cancer.

Safety and Tolerability

Adverse events (AEs) were recorded at the end of each treatment cycle and at the end of the study period using data from the safety population (all patients who received treatment at least once in the study). AEs were categorized according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) Version 2. The incidence of significant decreases in left ventricular ejection fraction (LVEF) and serious AEs were recorded. The CBC was evaluated weekly. Serum chemistries and urinalysis were evaluated every 2 weeks. The minimum hematology values and LVEF in each treatment cycle were also recorded and analyzed in the per-protocol set (PPS; all patients who received treatment at least once and had no protocol deviations).

Pharmacokinetics

To assess the effect of concomitant dofequidar use on the pharmacokinetics of doxorubicin, the plasma doxorubicin concentration on day 1 of cycle 1 was compared between treatment groups. Blood samples were taken at baseline and at 15 minutes, 30 minutes, and 1, 2, 4, and 6 hours after the start of doxorubicin administration. Plasma doxorubicin concentrations were determined by reversed-phase high-performance liquid chromatography. Area

under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal rule.

Statistical Analyses

The primary end point was analyzed using the Fisher's exact test at a significance level of 2.5% in a one-sided test. A difference in response rates of 20% between the two treatment groups was used as the basis for a statistically significant difference. CR, TTF, TTP and PFS were analyzed by the log-rank test at a significance level of 5% in a two-sided test. The CR, TTF, TTP and PFS were analyzed in the FAS, and the pharmacokinetic data analyzed in the PPS.

Patient Characteristics

A total of 227 patients were recruited onto the study (Fig A1, online only), of which 225 patients were included in the safety analysis (n = 113 for the dofequidar group; n = 112 for the placebo group); two patients did not receive the study treatment and were thus excluded. Four patients did not meet the inclusion/exclusion criteria; therefore, the FAS consisted of 221 patients (n = 113 for the dofequidar group; n = 108 for the placebo group). The PPS consisted of 199 patients (n = 100 for the dofequidar group; n = 99 for the placebo group). There were 22 patients excluded from the PPS analysis due to protocol deviations. Baseline patient characteristics were well balanced between the two treatment arms (Table 1). Most patients had predominantly recurrent disease and had received prior chemotherapy plus endocrine therapy. Also, many patients who had advanced primary breast cancer had received no prior therapy.

Table 1. Patient Demographics (full analysis set)

Characteristic	Dofequidar + CAF (n = 113)		Placebo + CAF (n = 108)	
	No.	%	No.	%
Age, years				
Mean	54.4		52.4	
SD	7.69		8.97	
Medical history known	65	57.5	60	55.6
Weight, kg				
Mean	56.2		54.1	
SD	7.52		7.73	
Height, cm				
Mean	154.7		154.7	
SD	5.71		5.61	
Body surface area, m^2				
Mean	1.5		1.5	
SD	0.11		0.11	
Disease state				
Recurrent	81	71.7	80	74.1
Advanced	32	28.3	28	25.9
Prior therapy				
Radiotherapy + chemotherapy + endocrine therapy	32	22.1	32	29.6
Chemotherapy + endocrine therapy	55	48.7	54	50.0
Radiotherapy	1	0.9	1	0.9
No prior therapy	25	22.1	21	19.4
Menopausal status				
Premenopausal	24	21.2	26	24.1
Postmenopausal	88	77.9	79	73.1

Abbreviations: CAF, cyclophosphamide, doxorubicin, and fluorouracil; SD, standard deviation.

Efficacy

The ORR, rated as CR or partial response rate, was 42.6% for CAF plus placebo versus 53.1% for dofequidar plus CAF (Table 2). Although this represents a 24.6% relative improvement and a 10.5% absolute increase in response rate for patients receiving dofequidar plus CAF compared with those receiving CAF plus placebo, this response was not statistically significant ($P = .077$). A higher value was observed in the dofequidar treatment group for all secondary end points compared with placebo, though these results were not statistically significant. Among them, Figure 2 shows a trend for prolonged PFS (median, 241 days for CAF plus placebo *v* 366 days for dofequidar plus CAF; $P = .145$).

Dofequidar plus CAF significantly improved PFS in several patient subgroups, including patients who were premenopausal ($P = .046$; Fig 3A), patients who had not received prior therapy ($P = .0007$; Fig 3B), and patients who had advanced primary breast cancer ($P = .017$; Fig 3C). An extended follow-up showed that dofequidar plus CAF also significantly improved overall survival ($P = .0034$; Fig 3D) in patients who had no prior therapy.

Safety and Tolerability

A similar number of patients completed six treatment cycles in both groups (n = 53 for the dofequidar group; n = 51 for the placebo group). The mean number of treatment cycles was 4.5 in the dofequidar group and 4.3 in the placebo group. More than half of patients in both groups included in each cycle from cycle 2 onward had a delay in treatment, mostly due to prolonged hematologic toxicities.

Dofequidar plus CAF was well tolerated throughout the study. No statistically significant excess of grade 3/4 AEs, except for neutropenia ($P = .006$) and leukopenia ($P = .005$), was found in the dofequidar group compared with placebo (Table A1, online only). Importantly, there was no marked difference in the incidence of neutropenia-related morbidity, such as febrile neutropenia or infection, between the two treatment groups. No significant differences in the incidence of cardiac AEs were found between the two treatment groups. In addition, dose intensities of chemotherapeutic agents were similar in both treatment arms. No significant difference in the incidence of serious AEs (SAEs) was observed between either group. However, there was a trend for a higher incidence of SAEs from leukopenia in the dofequidar group than in the placebo group ($P = .060$; Fisher's exact test); five leukopenia cases were reported for dofequidar, whereas no such case was reported for placebo.

A total of 124 patients discontinued the study (n = 61 for the dofequidar group; n = 63 for the placebo group). The major reasons for discontinuation were progressive disease (n = 23 for the dofequidar group; n = 28 for the placebo group), grade 4 hematologic toxicity (n = 20 for the dofequidar group; n = 6 for the placebo group), failure to meet treatment continuation criteria (n = 6 for the dofequidar group; n = 8 for the placebo group), and consent withdrawal (n = 6 for the dofequidar group; n = 12 for the placebo group). Of the 225 patients who received treatment in the study, 14 patients died during the treatment period (n = 3), the follow-up period (n = 2), or the follow-up period after study termination (n = 9). There were 49 other serious AEs in 32 patients during the study and follow-up period.

Pharmacokinetics

The mean plasma concentrations of doxorubicin in the dofequidar- and placebo-treatment groups at 15 minutes postadministration reached 0.997 μg/mL and 1.259 μg/mL, respectively, followed by biphasic elimination in both treatment groups. Mean plasma concentrations in

Table 2. Response Rates for Patients Treated With Dofequidar Plus CAF (n = 113) or Placebo Plus CAF (n = 108)

Treatment Group	Parameter (No. of patients)					Overall Response Rate (%)	95% CI
	Complete Response	Partial Response	No Change (stable disease)	Progressive Disease	Not Assessable		
Dofequidar	5	55	40	10	3	53.1	43.5 to 62.5
Placebo	4	42	41	14	7	42.6	33.1 to 52.5

NOTE. Odds ratio = 1.53 (range, 0.87-2.69); P = .077 for dofequidar *v* placebo.
Abbreviation: CAF, cyclophosphamide, doxorubicin, and fluorouracil.

the dofequidar and placebo groups remained similar at 1, 2, 4, and 6 hours after the start of doxorubicin administration. Thus the elimination pattern for the first 6 hours after the start of administration was similar in both groups. The plasma concentrations of doxorubicin in the terminal phase (4 and 6 hours postadministration) were slightly higher in the dofequidar group compared with placebo (1.2- to 1.3-fold). However, AUC (0 to 6 hours) values showed no statistically significant difference between the dofequidar and placebo groups (mean, 0.480 $\mu g \cdot h/mL$; standard deviation [SD], 0.324; range, 0.237-1.692; and mean, 0.407 $\mu g \cdot h/mL$; SD, 0.062; and range, 0.289-0.500, respectively). Therefore, treatment with dofequidar did not affect the plasma concentrations of doxorubicin in patients (Fig 4).

Discussion

Chemotherapy remains the preferred adjuvant treatment for patients with hormone receptor–negative disease and for patients with more aggressive, hormone receptor–positive tumors.[11,20] However, despite the use of conventional adjuvant chemotherapy regimens, a significant proportion of patients with breast cancer still experience disease recurrence because of inherent or acquired drug resistance.[12] In this randomized phase III trial, the efficacy and safety of the multidrug resistance inhibitor dofequidar plus CAF was compared with CAF plus placebo in patients with recurrent or advanced breast cancer. Although, there was an observed relative improvement and absolute increase in response rate for patients who received dofequidar plus CAF, these results did not reach statistical significance. This improvement in response rate may have been reflected in the observation that there was a trend for prolonged PFS, which favored patients in the dofequidar plus CAF group.

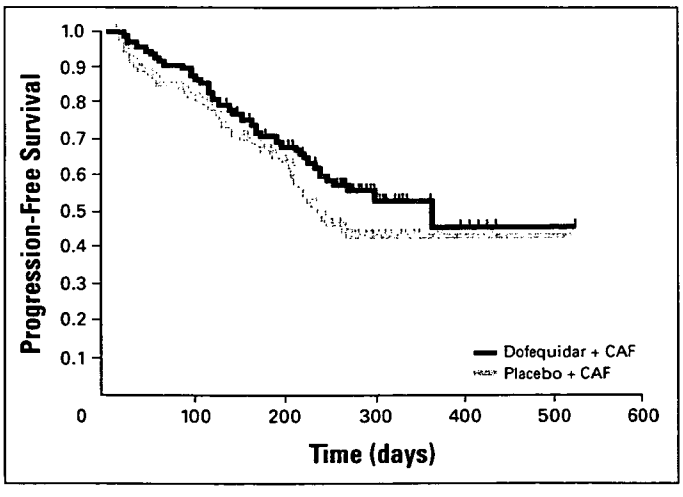


Fig 2. Progression-free survival in patients treated with dofequidar plus cyclophosphamide, doxorubicin, and fluorouracil (CAF) and placebo plus CAF (P = .145).

To date, only two randomized trials have examined the efficacy of a P-gp inhibitor in combination with chemotherapy in breast cancer patients. Wishart et al[21] examined quinidine combined with epirubicin in patients with advanced breast cancer, but failed to show any significant difference in overall survival or PFS compared with placebo. In a more recent prospective study of patients with anthracycline-resistant metastatic breast cancer (n = 99), verapamil combined with vindesine and fluorouracil resulted in a significantly longer overall survival and a higher response rate compared with patients who did not receive the P-gp inhibitor (median survival, 323 *v* 209 days; P = .036, respectively; ORR, 27% *v* 11%; P = .04, respectively).[22]

In the subgroup analyses, dofequidar in combination with CAF displayed a significantly increased PFS in patients who had not received prior therapy, who had advanced primary breast cancer or who were premenopausal. In addition, dofequidar also significantly improved overall survival in the patient group who had no prior therapy. Although the patient numbers in these analyses were small, the results remain important within these clinically significant patient populations. Both preclinical and clinical data have indicated that newer-generation MDR modulators can prevent the development of resistance.[23,24] A phase I/II trial in patients with acute myeloid leukemia showed that dosing with cyclosporine before and in combination with daunorubicin prevented chemotherapy resistance, while also resulting in a decrease in MDR-1 RNA expression.[24] Our results may highlight one potential treatment approach to MDR tumors that has not yet been fully exploited in the clinical environment, specifically the prevention of the emergence of resistance through the early use of P-gp inhibitors.[1-3] It seems reasonable that agents such as dofequidar may be useful in the adjuvant or even neoadjuvant setting with the goal of preventing or delaying the induction of MDR associated with chemotherapy.

The potential clinical significance of P-gp and MRP expression in breast cancer is supported by the results from a number of studies. For example in a study of primary breast cancer patients (n = 259), MRP expression was associated with an increased risk of treatment failure in patients with small tumors (T1) and node-positive patients who received adjuvant cyclophosphamide, methotrexate, and fluorouracil (CMF) chemotherapy but not in node-negative patients.[25] Burger et al[12] reported that the expression of MDR1 mRNA in primary breast tumors was inversely correlated with the efficacy of first-line chemotherapy. Additionally, the high level of MDR1 expression was suggested to be a significant predictor of poor prognosis in patients

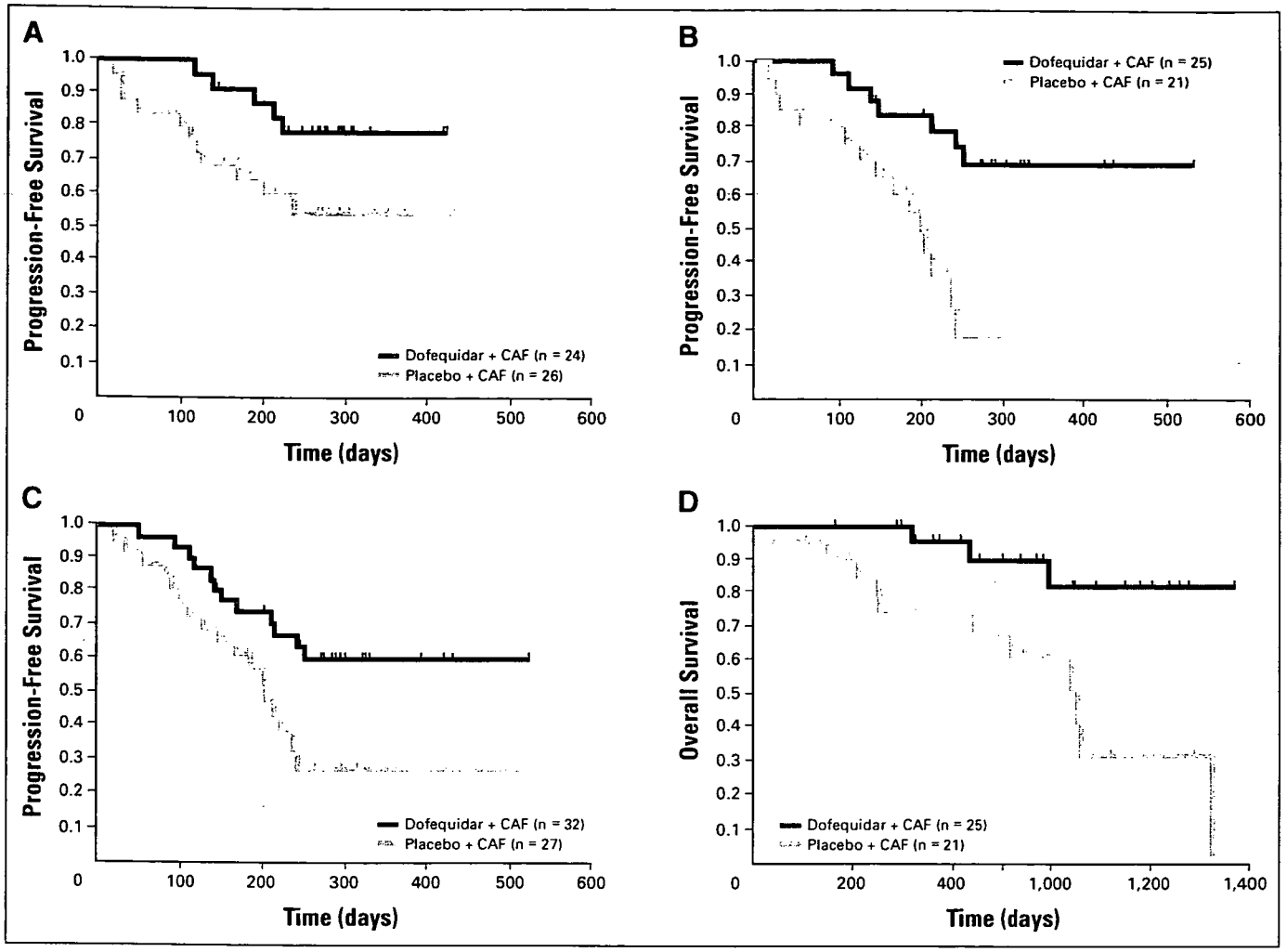


Fig 3. Subgroup analyses. (A) Progression-free survival in premenopausal patients (P = .046); (B) progression-free survival in patients who had no prior therapy (P = .0007); (C) progression-free survival in patients who were stage IV at diagnosis with an intact primary tumor (P = .017); and (D) overall survival in patients who had no prior therapy (P = .0034).

with advanced disease.[12] Significantly increased expression of P-gp and MRP-1 has also been reported in an immunohistochemical study of patients treated with preoperative chemotherapy, whereas pretreatment expression of MRP-1 was associated with significantly shorter PFS in patients.[26] In a more recent study, MRP-1 expression was shown to be an independent predictor for shorter relapse-free survival and overall survival, after adjuvant CMF treatment, in premenopausal, hormone receptor–positive patients.[27] However, MRP-1 expression did not affect patients' response to adjuvant tamoxifen plus goserelin treatment.[27]

These findings and our results support the view of Leonard et al,[3] who indicate that future patients will need to be carefully selected for the identification and development of effective drug-resistance modulators. Patient populations who may derive maximal benefit from MDR inhibition, for example, the no-prior-therapy, advanced-disease, or premenopausal patient group in the present study, could quite easily be overlooked or lost within a large, heterogeneous trial population.[3] Furthermore, by refining future clinical trials to incorporate specific disease and patient characteristics, a clearer picture of drug resistance in cancer will be obtained and the most effective MDR inhibitor/chemotherapeutic agent(s) selected.

Many MDR inhibitors have required high serum concentrations for MDR reversal, which resulted in unacceptable toxicity, thereby limiting their clinical impact.[7,28-32] Although more recent agents have shown improved tolerability profiles, this has been countered by unpredictable pharmacokinetic interactions with other transporter molecules (eg, cytochrome P450–mediated drug metabolism and excretion, necessitating dose reductions in chemotherapy agents and leading to inconsistent chemotherapy dosing among patients).[1,5] Similarly, the addition of the MDR-modulating agent valspodar (PSC 833) to chemotherapy agents did not improve treatment outcome.[33,34] Toxicity was increased in the valspodar-treated group compared with chemotherapy agents alone, despite the reduction of chemotherapy doses in the valspodar-containing regimen. In our study, dofequidar was well tolerated, with no indication of the unacceptable toxicity associated with early MDR inhibitors. Importantly, dofequidar did not affect the plasma concentrations of doxorubicin in patients during the study and displayed an acceptable pharmacokinetic profile.

In conclusion, this study suggests that treatment with dofequidar resulted in possible clinical benefit for patients who had not received prior therapy, who were premenopausal, or who were stage IV at diagnosis with an intact primary tumor. Dofequidar was also well

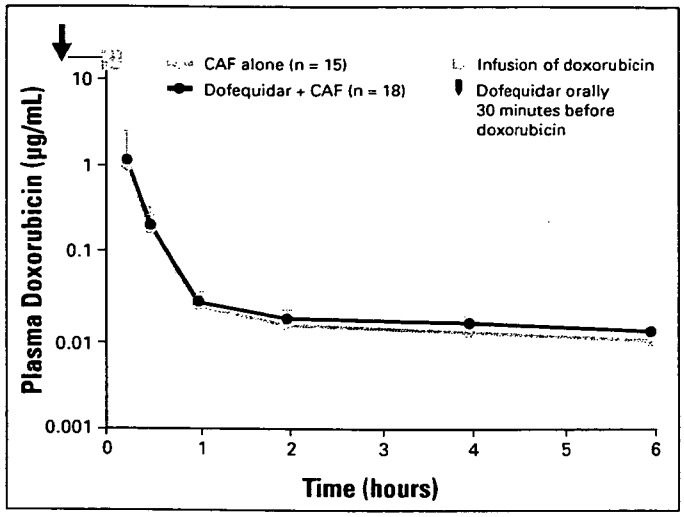


Fig 4. Plasma levels of doxorubicin in patients receiving dofequidar or placebo. CAF, cyclophosphamide, doxorubicin, and fluorouracil.

tolerated in the clinical setting and had no impact on doxorubicin pharmacokinetics. Further studies are merited to assess the effect of dofequidar in specific patient populations with breast cancer.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author or immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment: N/A **Leadership:** N/A **Consultant:** Toshiaki Saeki, Nihon Schering; Takashi Tsuruo, Nihon Schering **Stock:** N/A **Honoraria:** N/A **Research Funds:** N/A **Testimony:** N/A **Other:** N/A

AUTHOR CONTRIBUTIONS

Conception and design: Toshiaki Saeki, Masakazu Toi, Yoshinori Ito, Shinzaburo Noguchi, Tadashi Kobayashi, Hironobu Minami, Tadashi Ikeda, Yasuo Ohashi, Wakao Sato, Takashi Tsuruo

Financial support: Wakao Sato

Administrative support: Toshiaki Saeki, Tadashi Ikeda

Provision of study materials or patients: Toshiaki Saeki, Tadashi Nomizu, Masakazu Toi, Yoshinori Ito, Shinzaburo Noguchi, Tadashi Kobayashi, Taro Asaga, Hironobu Minami, Naohito Yamamoto, Kenjiro Aogi, Tadashi Ikeda

Collection and assembly of data: Toshiaki Saeki, Tadashi Nomizu, Masakazu Toi, Yoshinori Ito, Shinzaburo Noguchi, Tadashi Kobayashi, Taro Asaga, Hironobu Minami, Naohito Yamamoto, Kenjiro Aogi

Data analysis and interpretation: Toshiaki Saeki, Masakazu Toi, Yoshinori Ito, Shinzaburo Noguchi, Tadashi Kobayashi, Hironobu Minami, Tadashi Ikeda, Yasuo Ohashi, Wakao Sato

Manuscript writing: Toshiaki Saeki, Wakao Sato

Final approval of manuscript: Toshiaki Saeki, Tadashi Nomizu, Masakazu Toi, Yoshinori Ito, Shinzaburo Noguchi, Tadashi Kobayashi, Taro Asaga, Hironobu Minami, Naohito Yamamoto, Kenjiro Aogi, Tadashi Ikeda, Yasuo Ohashi, Wakao Sato, Takashi Tsuruo

REFERENCES

1. Gottesman MM, Fojo T, Bates SE: Multidrug resistance in cancer: Role of ATP-dependent transporters. Nat Rev Cancer 2:48-58, 2002
2. Loe DW, Deeley RG, Cole SPC: Biology of the multidrug resistance-associated protein, MRP. Eur J Cancer 32A:945-957, 1996
3. Leonard GD, Fojo T, Bates SE: The role of ABC transporters in clinical practice. Oncologist 8:411-424, 2003
4. Michalak K, Hendrich AB, Wesolowska O, et al: Compounds that modulate multidrug resistance in cancer cells. Cell Biol Mol Lett 6:362-368, 2001
5. Thomas H, Coley HM: Overcoming multidrug resistance in cancer: An update on the clinical strategy of inhibiting p-glycoprotein. Cancer Control 10: 159-165, 2003
6. Ambudkar SV, Dey S, Hrycyna CA, et al: Biochemical, cellular, and pharmacological aspects of the multidrug transporter. Annu Rev Pharmacol Toxicol 39:361-398, 1999
7. Krishna R, Mayer LD: Multidrug resistance (MDR) in cancer: Mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. Eur J Pharm Sci 11:265-283, 2000
8. Mechetner E, Kyshtoobayeva A, Zonis S, et al: Levels of multidrug resistance (MDR1) P-glycoprotein expression by human breast cancer correlate with *in vitro* resistance to taxol and doxorubicin. Clin Cancer Res 4:389-398, 1998
9. Esteva FJ, Valero V, Pusztai L, et al: Chemotherapy of metastatic breast cancer: What to expect in 2001 and beyond. Oncologist 6:133-146, 2001
10. Hortobagyi GN: Treatment of breast cancer. N Engl J Med 339:974-984, 1998
11. National Comprehensive Cancer Network: Clinical Practice Guidelines in Oncology, version 1. Jenkintown, PA, National Comprehensive Cancer Network, 2005
12. Burger H, Foekens JA, Look MP, et al: RNA expression of breast cancer resistance protein, lung resistance-related protein, multidrug resistance-associated proteins 1 and 2, and multidrug resistance gene 1 in breast cancer: Correlation with chemotherapeutic response. Clin Cancer Res 9:827-836, 2003
13. Kroger N, Achterrath W, Hegewisch-Becker S, et al: Current options in treatment of anthracycline-resistant breast cancer. Cancer Treat Rev 25:279-291, 1999
14. Tsuruo T, Iida H, Tsukagoshi S, et al: Overcoming of vincristine resistance in P388 leukemia in vivo and in vitro through enhanced cytotoxicity of vincristine and vinblastine by verapamil. Cancer Res 41:1967-1972, 1981
15. Tsuruo T: Circumvention of drug resistance with calcium channel blockers and monoclonal antibodies, in Ozols R (ed): Drug Resistance in Cancer Therapy. Norwell, MA, Kluwer Academic Publishers, 1989, pp 73-95
16. Tsuruo T, Naito M, Tomida A, et al: Molecular targeting therapy of cancer: Drug resistance, apoptosis and survival signal. Cancer Sci 94:15-21, 2003
17. Sato W, Fukazawa N, Nakanishi O, et al: Reversal of multidrug resistance by a novel quinoline derivative, MS-209. Cancer Chemother Pharmacol 35:271-277, 1995
18. Nakanishi O, Baba M, Saito A, et al: Potentiation of the antitumor activity by a novel quinoline compound, MS-209, in multidrug-resistant solid tumor cell lines. Oncol Res 9:61-69, 1997
19. Narasaki F, Oka M, Fukuda M, et al: A novel quinoline derivative, MS-209, overcomes drug resistance of human lung cancer cells expressing the multidrug resistance-associated protein (MRP) gene. Cancer Chemother Pharmacol 40:425-432, 1997
20. Margolese RG, Hortobagyi GN, Bucholz TA: Neoplasms of the breast, in Kufe DW, Pollock RE, Weichselbaum RR, et al (eds): Cancer Medicine, (ed 6). Hamilton, Canada, BC Decker Inc, 2003
21. Wishart GC, Bissett D, Paul J, et al: Quinidine as a resistance modulator of epirubicin in advanced breast cancer: Mature results of a placebo-controlled randomized trial. J Clin Oncol 12:1771-1777, 1994
22. Belpomme D, Gauthier S, Pujade-Lauraine E, et al: Verapamil increases the survival of patients with anthracycline-resistant metastatic breast carcinoma. Ann Oncol 11:1471-1476, 2000
23. Cocker HA, Tiffin N, Pritchard-Jones K, et al: In vitro prevention of the emergence of multidrug resistance in a pediatric rhabdomyosarcoma cell line. Clin Cancer Res 7:3193-3198, 2001
24. List AF, Spier C, Greer J, et al: Phase I/II trial of cyclosporine as a chemotherapy-resistance