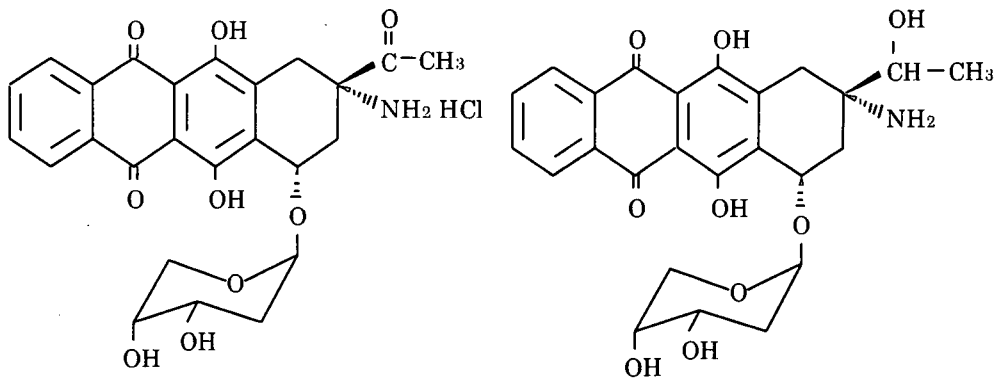

Fig. 1 Chemical structures of amrubicin hydrochloride (*left*) and amrubicinol (*right*)

125 mg/m^2. However, because a clear tumor shrinking effect was not seen in any subject in this study,[8] subsequent repeated 5-day administration studies were not carried out. On the other hand, Feld et al. performed a clinical study of another anthracycline antitumor drug, epirubicin, for the treatment of non-small-cell lung cancer (NSCLC), and reported higher response rate in three-consecutive-day administration than in single-dose administration [9]. Based on these findings and in consideration of convenience in practical therapy, a regimen of repeated doses for three consecutive days came to be recommended for amrubicin as well. This article reviews the clinical studies of amrubicin for the treatment of NSCLC and small-cell lung cancer (SCLC) that have already been completed and suggests a course for investigations in the future.

Non-small-cell lung cancer

Two single-dose phase II clinical studies of amrubicin for the treatment of NSCLC were conducted. First, an early phase II study targeted previously untreated NSCLC, starting with a dose of 100 mg/m^2 every 3 weeks. Adverse events in 16 subjects initially enrolled were mild, and the study was therefore continued in additional 26 subjects at an increased dose of 120 mg/m^2. Among 14 evaluable subjects of the initial 16, 1 subject (7.1%) had a partial response (PR), and among 20 evaluable subjects of the additional 26 after dose increase, 5 subjects (25%) had PR. Following these promising results, a late phase-II study was conducted for previously untreated NSCLC at a dose of 120 mg/m^2. A total of 62 patients were enrolled, but contrary to expectations only 6 subjects had PR, for an overall response rate of 9.7% [fn: New Drug Approval Package (in Japanese) http://www.info.pmda.go.jp/shinyaku/g020402/37009000_21400AMZ00465_x100_1.pdf, p501–510, p517–523, p524–532].

Prior to these studies, no phase I studies involving the recommended course of repeated administration over 3 days had been performed. Therefore, a phase I/II study on previously untreated NSCLC was conducted [10]. A dosage of 40 mg/m^2/day (total dose of 120 mg/m^2) was established for level 1, and was increased to 45 and 50 mg/m^2/day for levels 2 and 3, respectively. Four patients each were enrolled at dosage levels 1 and 2, and 5 patients at level 3 [10]. At level 3, grade 4 adverse events persisting 4 days or longer were leukopenia in two of five subjects and neutropenia in five of five subjects. Adverse events higher than grade 3 were thrombocytopenia in two of five subjects and anemia in two of five subjects. Non-hematologic grade 3 adverse events seen in one subject each were nausea/vomiting and melena. Grade 4 hematemesis was also seen in one subject. The DLTs were leukopenia, neutropenia, thrombocytopenia, and gastrointestinal disturbances, and so 50 mg/m^2 was considered to be the MTD [10]. The recommended dosage for phase II studies was considered to be 45 mg/m^2/day. Additional 15 evaluable patients were registered for the study at this dosage, and 7 of the total 28 subjects had PR, with an overall response rate of 25%. These results of amrubicin monotherapy for NSCLC were essentially as promising as the results for other novel

Table 1 Effects of multiple administrations of amrubicin on the growth of human tumor xenografts

Dose	Schedule	Minimum T/C (%)							
		Lung carcinoma		Stomach carcinoma					
		LX-1	QG-56	SC-2	SC-7	SC-9	St-4	St-15	4-1ST
25 mg/kg	Once	43	44	46	59	59	29	39	11
7.5 mg/kg	5 qd	31[a]	38	36	37	37	29	24[a]	13

[a] 7.5 mg/kg daily for 5 days shows significantly superior growth inhibition over single 25 mg/kg dose ($p<0.05$)

antitumor drugs, such as paclitaxel, launched in the 1990s [10]. Additional phase II studies were conducted to further ascertain efficacy and safety, at a dosage of 45 mg/m^2/day for three consecutive days every 3 weeks (Table 2) [11]. A total of 61 patients (45 males) were enrolled (median age, 65 years; range, 33 to 75 years), and the majority of subjects had a performance status (PS) of 0 to 1. All subjects were evaluable for both efficacy and safety. One subject had a complete response (CR) and 16 subjects had PR, with an overall response rate of 27.9%. Among toxicities, hematologic toxicities were observed frequently. Higher than grade 3 leukopenia and thrombocytopenia were seen in 52.5 and 14.8% of the subjects, respectively. Neutropenia was seen in 72.1%, and anemia in 23.0%. Non-hematologic adverse events were mild, including higher than grade 3 nausea/vomiting in 4.9% and anorexia in 4.9% (Table 3). In three subjects, interstitial pneumonitis that had developed before enrollment was exacerbated during the study, and two of these subjects died. The median survival time (MST) was 11.3 months and 1-year survival rate 47.7% (Table 4) [11]. These results of overall response rates and survival are comparable to those achieved with standard two-drug combination therapy containing a platinum agent for advanced NSCLC. At present, results of clinical trials of combination therapy using amrubicin plus other drugs to evaluate effects on NSCLC have not yet been reported. It is urgent that we explore combination therapy using amrubicin with other drugs that are known to be effective in the treatment of NSCLC, but it is also important that we clarify the position of amrubicin in the practical treatment of NSCLC.

Small-cell lung cancer

A single-dose phase II study of amrubicin in patients with SCLC, previously treated or untreated, was performed similarly to the NSCLC studies. The dose was started at 100 mg/m^2 and increased to 120 mg/m^2 during the study. Eleven patients were enrolled (7 at 100 mg/m^2), of whom ten had previously been treated. Two of the 6 evaluable subjects treated with 100 mg/m^2 had PR, but no response was seen in any of the 4 subjects treated with 120 mg/m^2. Overall, 2 of the ten subjects had PR, for a response rate of 20%. The main adverse event was myelotoxicity. Grade 4 thrombocytopenia was seen in 4 of the 11 subjects (3 treated with 100 mg/m^2). In order to ascertain the efficacy of amrubicin in SCLC more accurately, a late phase II study in previously untreated patients with advanced SCLC was conducted at a dosage of 45 mg/m^2/day for three consecutive days at 3-week intervals. From an ethical standpoint, this study was designed such that if a tumor shrinkage of 25% or more (measured bilaterally) after one course, or 50% or more after two courses of amrubicin was not obtained, the patient would immediately be switched to the standard therapeutic mode of a combination of cisplatin and etoposide. A total of 35 patients were enrolled, and among the 33 evaluable subjects 3 had CR and 22 had PR, for an overall response rate of 75.8% (CR rate 9.1%). The MST was 11.7 months, the 1-year survival rate 48.5%, and the 2-year survival rate 20.2% (Table 4) [12]. Because a promising result of monotherapy had been obtained, a phase I/II combination therapy clinical study for previously untreated advanced SCLC was performed using cisplatin, a drug that currently plays a central role in SCLC chemotherapy, and the results were reported by Ohe et al [13]. In level 1, the dosage of amrubicin was 40 mg/m^2/day for three consecutive days, and the dose of cisplatin was 60 mg/m^2 (day 1); in levels 2 and 3 the dosage of amrubicin was 45 mg/m^2/day and the doses of cisplatin were 60 mg/m^2 and 80 mg/m^2, respectively. The courses were administered at 3-week intervals. DLTs, consisting of febrile neutropenia, grade 4 neutropenia persisting 4 days or more, and constipation, were seen in all three subjects enrolled at level 2. Therefore, the dosages at level 2 were considered the MTD, and the recommended dosage for the phase II part of the study was determined to be 40 mg/m^2/day for amrubicin with 60 mg/m^2 cisplatin. Then the phase II study was conducted in 41 subjects at that recommended dosage.

Table 2 Phase II studies of amrubicin in previously untreated advanced NSCLC: patient characteristics

Characteristics	Value
No. of eligible patients	61
Sex (male/female)	45/16
Age, median years (range)	65 (33–75)
Histology (adenocarcinoma/squamous/large cell)	33/26/2
Stage (IIIA/IIIB/IV)	8/19/34
PS (0/1/2)	19/39/3
No. of institutions	16

Table 3 Phase II studies of amrubicin in previously untreated advanced NSCLC: toxicities

Toxicity	No. of patients	Frequency (%)	
		>Gr. 1	≥Gr. 3
Anemia	61	78.7	23.0
Leukopenia	61	91.8	52.5
Neutoropenia	61	96.7	72.1
Thrombocyopenia	61	44.3	14.8
Anorexia	61	70.5	4.9
Nausea/vomiting	61	57.4	4.9
Diarrhea	61	9.8	0
Alopecia	60	71.7	1.7

Table 4 Phase II study of amrubicin in previously untreated patients with lung cancer

Study	No. of eligible patients	Response	MST	1-yr survival	2-yr survival
NSCLC	61	27.9%	11.3 months	47.7%	26.5%
ED-SCLC	33	75.8%	11.7 months	48.5%	20.2%

NSCLC, non-small cell lung cancer
ED-SCLC, extensive disease-small cell lung cancer

The response rate was 87.8%, with a CR rate of 9.8%, and the MST and 1-year survival rate were reported to be 13.6 months and 56.1%, respectively [13]. With respect to the treatment status, 78% of the subjects were able to undergo 4 or more courses as scheduled, but there were nine subjects (22%) in whom treatment had to be terminated because no effect was seen in two patients and adverse events (gastric ulcer, neutropenia, thrombocytopenia, febrile neutropenia, hyponatremia, etc) occurred in seven patients. The dosage had to be decreased during treatment in 39 (23%) of the total 178 cycles. Almost all of the decreases involved a reduction in the dosage of amrubicin, to 30 mg/m^2/day in 12 (7%) of these cycles. Adverse events were higher than grade 3 leukopenia (65.9%), neutropenia (95.1%), thrombocytopenia (24.4%), and anemia (51.2%). Higher than grade 3 non-hematologic adverse events were anorexia (31.7%), nausea (19.5%), constipation (7.3%), vomiting (4.9%), and diarrhea (4.9%).

A recent Japanese study (Japan Clinical Oncology Group: JCOG 9511) comparing the combination of cisplatin and irinotecan hydrochloride (CPT-11) with the combination of cisplatin and etoposide in the treatment of ED-SCLC showed a significant advantage in overall survival favoring the combination of cisplatin/CPT-11 [14]. As the results obtained in this phase I/II study of the combination of cisplatin and amrubicin may be equal to or better than the results of cisplatin/CPT-11 combination therapy, JCOG is planning a randomized phase III study to compare the combinations of cisplatin/amrubicin and cisplatin/CPT-11 therapy for previously untreated ED-SCLC.

Relapsed SCLC

While amrubicin monotherapy was highly effective for previously untreated SCLC, no study had been conducted to evaluate the efficacy in the treatment of relapsed SCLC. As such, a phase II study was conducted in patients with relapsed disease who had previously received one or two regimens including at least one regimen of platinum-based chemotherapy [15]. Sixty patients were enrolled in this multicenter study, comprising 44 sensitive cases in which CR or PR was observed with the previous chemotherapy and the disease was then shown to have progressed or relapsed at least 60 days after the final dosing in the previous chemotherapy, and 16 refractory cases in which the disease progressed within 60 days after the final dosing in the previous chemotherapy. In consideration of bone marrow exhaustion associated with the previous therapy, four or more courses of administration at the 40 mg/m^2 level for three consecutive days were repeated at 3-week intervals.

The response rate was 52% (95% CI: 38–65%). The progression-free survival, overall survival, and 1-year survival rate were 3.9 months, 11.2 months, and 44.1%, respectively. In sensitive cases, the response rate was 52% (95% CI: 37–67%), and the progression-free survival, overall survival, and 1-year survival rate were 4.2 months, 11.6 months, and 45.5%, respectively. In refractory cases, the response rate was 50% (95% CI: 25–75%), and the progression-free survival, overall survival, and 1-year survival rate were 2.6 months, 10.3 months, and 40.3%, respectively (Table 5) [15]. Common adverse events were hematologic toxicities, including grade 3–4 neutropenia (83.3%), leucopenia (70.0%), anemia (33.3%), thrombocytopenia (20.0%), and febrile neutropenia (5%). Non-hematologic adverse events included grade 3–4 anorexia (15%) and asthenia (15%) [15].

Based on the results of this study, the efficacy of monotherapy for relapsed SCLC was compared in the response rate. In sensitive cases, the response rate was highest 52% (23/44) with amrubicin, followed by 28% (18/63), 19% (9/47), 18% (30/168), and 17% (7/41) with irinotecan, docetaxel, topotecan, and vinorelbine, respectively: a promising result for amrubicin. In refractory cases, the response rate was highest 50% (8/16) with amrubicin, followed by 29% (7/24), 14% (5/38), 8% (6/75), 3% (1/28), and 0% (0/8) with paclitaxel, gemcitabine, topotecan, irinotecan, and vinorelbine, respectively (Table 6) [16]. The survival variables were compared with the results from a past study of topotecan [17]. The CR rate, PR rate, progression-free survival, and overall survival were 2.3%, 50%, 4.2 months, and 11.6 months in the amrubicin group, versus 0%, 24.3%, 3.3 months, and 6.3 months in the topotecan group, respectively, showing a favorable result of amrubicin.

Amrubicin showed a comparable response rate in sensitive and refractory cases; however, as the present study involved only Japanese patients, it is desirable to conduct clinical studies overseas to confirm the efficacy.

Table 5 Phase II study of amrubicin in relapsed case or refractory case with small lung cancer: Response

	Sensitive case	Refractory case	Total
No. of patients	44	16	60
CR	1	1	2
PR	22	7	29
SD	10	2	12
PD	11	6	17
Response rate (95% CI)	52% (37–68%)	50% (25–75%)	52% (38–65%)
Progression-free survival (95% CI)	4.2 months (3.6–5.3)	2.9 months (1.4–4.6)	3.9 months (3.4–4.6)
Median survival time (95% CI)	11.6 months (10.0–15.8)	10.3 months (4.8–∞)	11. months (10.0–13.2)
1-yr survival (95% CI)	45.5% (29.9–59.8)	40.3% (15.1–64.6)	44.1% (30.6–56.8)

∞: a symbol of infinite

Future directions

As noted above, little evidence has been published concerning the efficacy of amrubicin in the treatment of NSCLC or SCLC. Only amrubicin monotherapy has been investigated for NSCLC, and only combination therapy with cisplatin has been investigated for SCLC.

At present, platinum-based doublet chemotherapy is considered the standard treatment as 1st line chemotherapy for advanced NSCLC. Therefore, combination therapy with cisplatin in previously untreated patients with advanced NSCLC should be tested. Combination therapy with carboplatin, an analog of cisplatin that is often used instead of cisplatin because of its milder toxicity profile, should also be evaluated. However, in combination with carboplatin, it is necessary to note that hematologic toxicities overlap, and therefore studies should start from phase I to determine a recommended dosage. Combination therapies with paclitaxel, docetaxel, gemcitabine, vinorelbine, and CPT-11, novel anticancer agents that became available in the 1990s, should also be topics of investigation as non-platinum regimens. However, it is already known that anthracycline anticancer agents and taxane agents interact: for example, in combination therapy using paclitaxel plus doxorubicin, it has been reported that if paclitaxel is administered first, not only do the pharmacokinetics of doxorubicin change, but its toxicity is increased [18]. Because amrubicin is also an anthracycline agent, any investigation of combination therapy with a taxane agent in particular should involve a pharmacokinetics study. Recently, Masuda et al. conducted a combination phase I study of CPT-11 and amrubicin, which led to a recommended dosage of 60 mg/m^2 of CPT-11 on days 1and 8, and 25 mg/m^2 of amrubicin, days 1–3 every 3 weeks, the lowest dosage levels that had been tested in their study because of adverse events, including strong myelotoxicity [19]. Regardless of whether or not it is combined with a platinum drug, it is necessary to clarify whether amrubicin can become a viable first line chemotherapy candidate for advanced NSCLC in the future.

Table 6 Responses of the "3rd generation drug" in sensitive relapse and refractory disease[a]

	Responders/evaluable	
	Sensitive relapse	Refractory disease
Topotecan	18% (30/168)	8% (6/75)
Irinotecan	28% (18/63)	3% (1/28)
Docetaxel	19% (9/47)	
Paclitaxel		29% (7/24)
Gemcitabine		14% (5/38)
Vinorelbine	17% (7/41)	0% (0/8)
Amrubicin	52% (23/44)	50% (8/16)

[a] Glisson BS, Semin Oncol 30: 72–78, 2003

The second line treatment of NSCLC and 1st line treatment in elderly patients are in categories for which single-agent chemotherapy should be the recommended option. It is necessary to test amrubicin for these categories. To date, amrubicin has been approved and licensed for 3-day administration, but a phase I clinical study of this administration method has only been conducted in previously untreated patients, and there is still a problem concerning whether the recommended dosage of 45 mg/m^2/day is tolerable in previously treated patients, especially in light of its strong myelotoxicity. On this point, Okamoto et al. recently conducted a phase I study of amrubicin in previously treated patients with lung cancer, and reported a recommended phase II dosage of amrubicin at 35 mg/m^2/day for three consecutive days every 3 weeks [20].

For ED-SCLC, based on the good results obtained from combination therapy with cisplatin, a randomized phase III study should be carried out involving a comparison with cisplatin–CPT-11 combination therapy. Other anticancer drugs that should be investigated for combination therapy include carboplatin, as well as the topoisomerase I inhibitors CPT-11 and topotecan, which have recently been playing major roles in the treatment of SCLC. Because no standard treatment has yet been established for SCLC that

recurs after the initial treatment, a possible target of amrubicin monotherapy is previously treated SCLC.

The clinical studies suggested above should be conducted for both NSCLC and SCLC; however, because amrubicin is strongly myelotoxic, special consideration should be taken if these drugs are used in combination. From this viewpoint, it is also important to examine the pharmacokinetic profile of amrubicin. There is only one report by Matsunaga et al. regarding the pharmacokinetics of amrubicin and its active metabolite amrubicinol in patients with lung cancer [21]. In this report, it was suggested that the area-under-the time curves of amrubicin and amrubicinol seemed to be associated with the hematologic toxicities, and interestingly interpatient variability in the enzymatic conversion of amrubicin to amrubicinol was small whereas a large interpatient variability in the clearance of amrubicin was observed [21].

Conclusion

Clinical studies of the novel anticancer agent amrubicin have only begun, and we as yet have little evidence to evaluate. However, there are high expectations for this agent in the trial to improve outcome for both NSCLC and SCLC patients. Many issues remain to be resolved, such as how to position this drug in the actual treatment of lung cancer. In order to resolve this and other issues in the future, many high-quality clinical studies are needed.

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ORIGINAL REPORT

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

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ABSTRACT

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.

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INTRODUCTION

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.

PATIENTS AND METHODS

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.

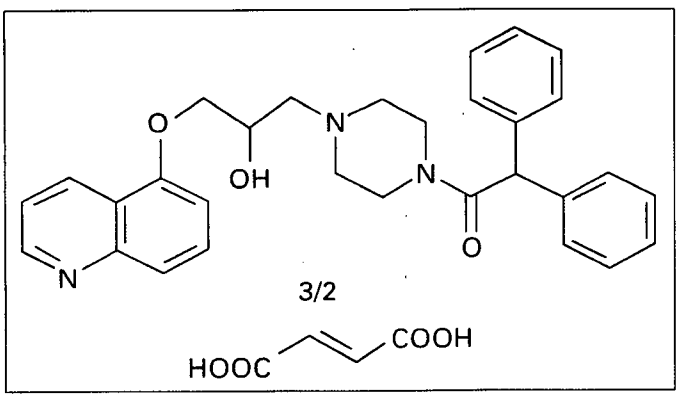

Fig 1. Structure of dofequidar (MS-209).

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.

RESULTS

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.

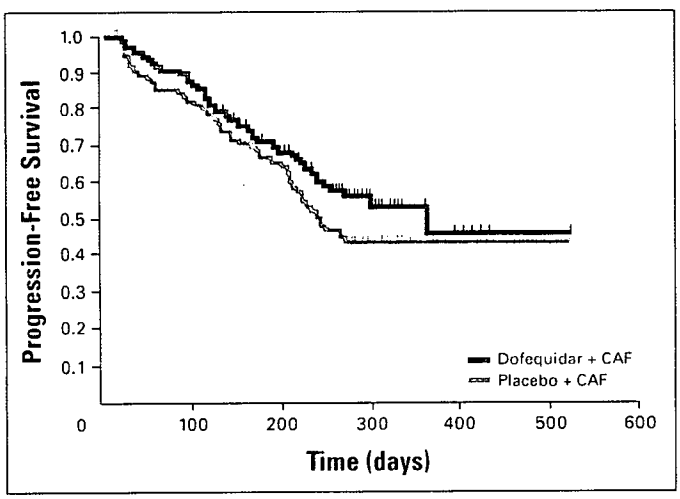

Fig 2. Progression-free survival in patients treated with dofequidar plus cyclophosphamide, doxorubicin, and fluorouracil (CAF) and placebo plus CAF (P = .145).

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

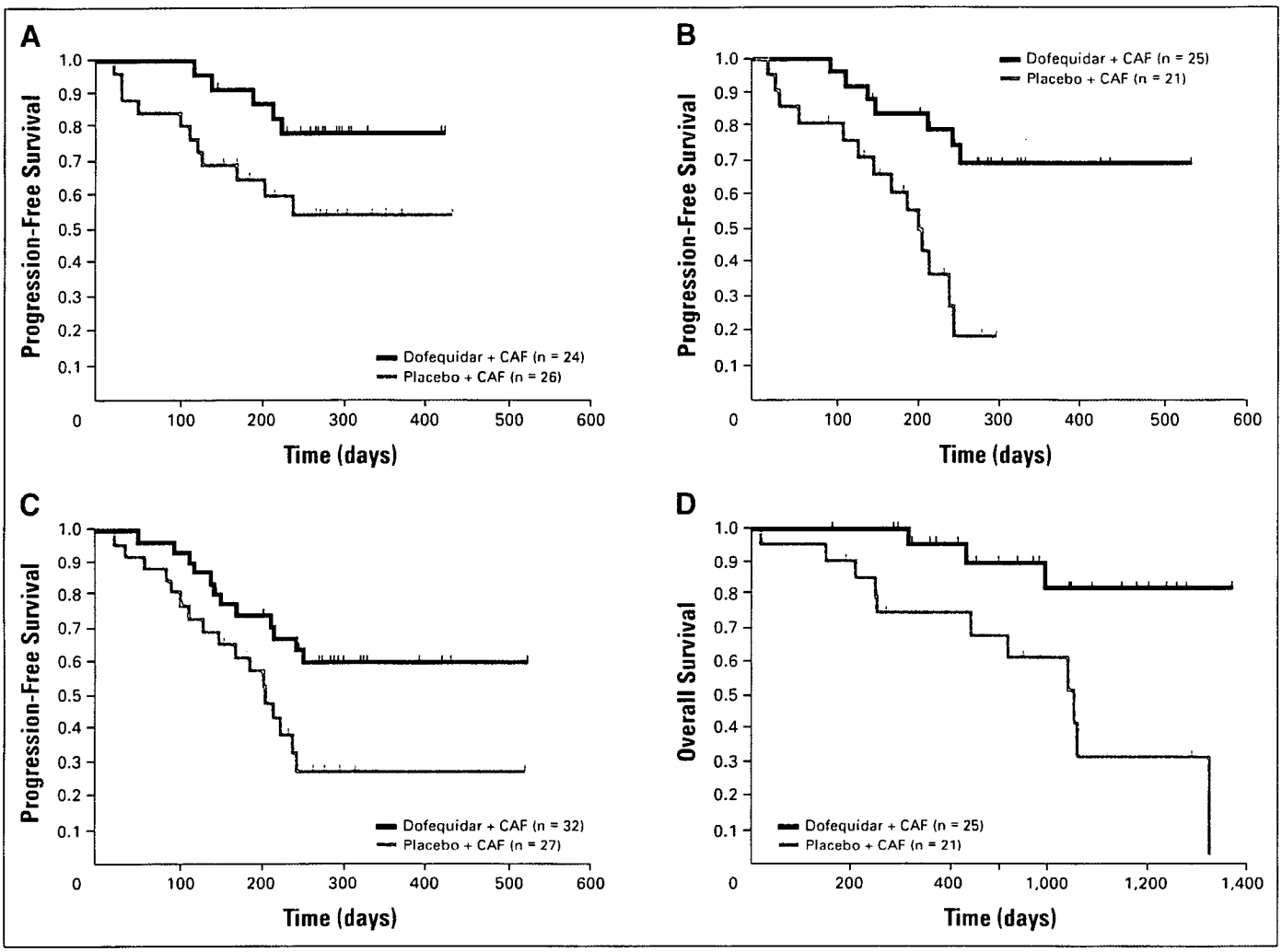

Fig 3. Subgroup analyses. (A) Progression-free survival in premenopausal patients (P = .046); (B) progression-free survival in patients who had no prior therapy (P = .0007); (C) progression-free survival in patients who were stage IV at diagnosis with an intact primary tumor (P = .017); and (D) overall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

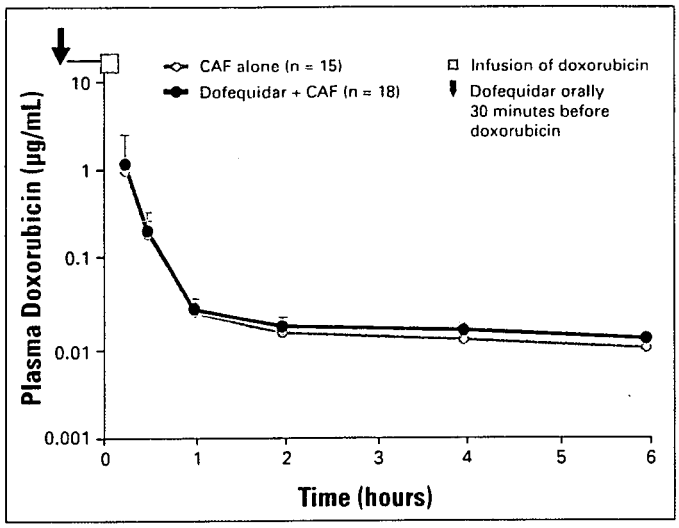

Fig 4. Plasma levels of doxorubicin in patients receiving dofequidar or placebo. CAF, cyclophosphamide, doxorubicin, and fluorouracil.

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.

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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Vol. 14 No. 1 January 2007

Review Article

Sentinel Lymph Node Biopsy is Feasible for Breast Cancer Patients after Neoadjuvant Chemotherapy

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Division of Surgical Oncology National Cancer Center Hospital

Background: Despite the increasing use of both sentinel lymph node (SLN) biopsy and neoadjuvant chemotherapy (NAC) in patients with operable breast cancer, information on the feasibility and accuracy of sentinel node biopsy following neoadjuvant chemotherapy is still quite limited. Therefore, we investigated the feasibility and accuracy of sentinel lymph node biopsy for breast cancer patients after NAC.

Methods: A total of 104 patients with Stage II and III breast cancers, previously treated by NAC, were enrolled in the study. All patients were clinically node-negative after NAC. The patients underwent SLN biopsy, which involved a combination of an intradermal injection of radiocolloid and a subareolar injection of blue dye over the tumor. This was followed by completion axillary lymph node dissection (ALND).

Results: SLN could be identified in 97 of 104 patients (identification rate, 93.3%). In 93 of the 97 patients (95.9%), the SLN accurately predicted the axillary status. Four patients' SLN biopsies were false negative, resulting in a false-negative rate of 10.0%. The SLN identification rate tended to be lower among patients with T4 primary tumors prior to NAC (62.5%).

Conclusion: The SLN identification and false-negative rates were similar to rates in non-neoadjuvant studies. The SLN accurately predicted metastatic disease in the axilla of patients with tumor response following NAC.

Breast Cancer 14:10-15, 2007.

Key words: Sentinel node biopsy, Neoadjuvant chemotherapy, Breast cancer, Intradermal injection

Introduction

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

Recent studies report identification rates greater than 90% and 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 have been established, including T3 or T4 tumors, multicentric or multifocal lesions, a large biopsy cavity, previous axillary surgery, previous chest-wall irradiation, and NAC[4,5].

The application of SLN biopsy in NAC patients may identify, as in non-neoadjuvant chemotherapy groups, patients who do not necessarily require an ALND. Several studies have evaluated the use of SLN biopsy in patients with breast cancer after NAC, but the results have been varied and inconclusive[6-14].

Recently, the American Society of Clinical Oncology panel concluded that there are insufficient data to recommend SLN biopsy for patients receiving preoperative therapy, although SLN biopsy after preoperative systemic chemotherapy is technically feasible[15]. It is possible that the tumor response to chemotherapy may alter or

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Abbreviations:
SLN, Sentinel lymph node; NAC, Neoadjuvant chemotherapy; ALND, Axillary lymph node dissection

interrupt the lymphatic drainage, thus causing lower SLN identification rates and higher false-negative rates than in non-neoadjuvant studies. We hypothesize that the lymphatic flow within the skin lesion overlying the tumor is less damaged by chemotherapy than that in the parenchyma surrounding the tumor, except in T4 tumors. Thus, the usefulness of SLN biopsy with intradermal radiocolloid injection for patients with NAC-treated breast cancer has yet to be established.

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

Patients and Methods

Between May 2003 and October 2005, 104 patients with T2-4N0-2 breast cancer underwent NAC with SLN biopsy plus ALND performed by a single surgeon. The pathologic diagnosis was established by core needle biopsy in all patients prior to NAC.

Patients under 65 of age received four cycles of 5FU (500mg/m^2) / epirubicin (100mg/m^2) / cyclophosphamide (500mg/m^2) (FEC), plus twelve weekly cycles of paclitaxel (80mg/m^2). Patients over 65 years of age received twelve weekly cycles of paclitaxel (80mg/m^2) alone. After NAC, we enrolled the 104 clinically node-negative patients into this study.

Lymphatic mapping was performed using a 3 ml combination of blue dye (Patent blue V®, TOC Ltd., Tokyo, Japan) and 30-80 megabecquerels of technetium-99m-labeled Phytate (Daiichi RI Laboratory, Tokyo, Japan). One day prior to surgery, the radiotracer was intradermally injected into the area overlying the tumor, while blue dye was intraoperatively injected into the subareolar site. For nonpalpable lesions, injections were performed using mammographic or ultrasonic needle localization. Sentinel lymph nodes were identified as blue stained, radioactive, or both. SLN biopsy was then followed by a standard level I/II ALND. For 32 patients, lymphscintigraphy was also performed prior to NAC, and was compared to lymphatic mapping after NAC.

All sentinel nodes were histologically evaluated by creating 3-5 mm serial sections and staining with hematoxylin and eosin (H&E). Lymph nodes submitted as part of the axillary dissection were submitted in their entirety and evaluated using standard H&E staining.

Table 1. Patient demographics

	Number of patients
Age (years)	
Mean	50.2
Range	27-77
Clinical tumor size (cm)*	
Mean	4.89
Range	2.5-12
Tumor classification*	
T2	61 (58.7%)
T3	35 (33.6%)
T4	8 (7.7%)
Lymph node status*	
N0	54 (52.0%)
N1	40 (38.5%)
N2	10 (9.5%)
Tumor type	
Invasive ductal	102 (98.1%)
Invasive lobular	2 (1.9%)
Type of NAC	
FEC plus paclitaxel	100 (96.2%)
paclitaxel alone	4 (3.8%)
Clinical response of the tumor	
CR	55 (52.9%)
PR	41 (39.4%)
SD	8 (7.7%)
Pathological response of the tumor	
pCR	23 (22.1%)
pINV	81 (77.9%)
Pathological nodal status	
Negative	60 (57.7%)
Positive	44 (42.3%)

*Before NAC.
pCR = pathological complete response; pINV = pathological invasive.
CR = Complete response; PR = Partial response; SD= Stable disease

Results

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

Based on lymphscintigraphy studies before and after NAC, the results of lymphatic mapping were quite similar in 30/32 patients, as shown in Fig 1. SLN were not detected in two cases with a

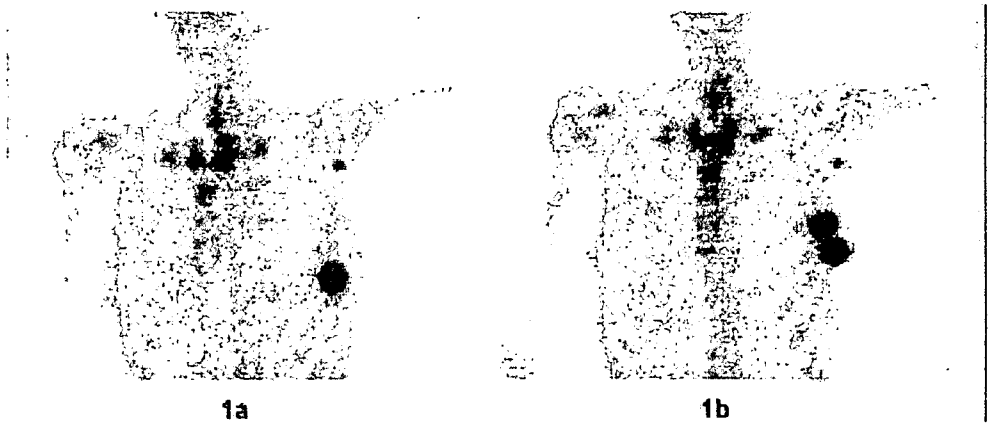

Fig 1. Lymphscintigraphy before and after NAC (1a and 1b, respectively) revealed one sentinel node at the axilla. The bone scintigram was performed simultaneously to detect bone metastasis.

Table 2. Results of sentinel node biopsy

	Number of patients
Total no. of patients	104
SLN identified	97 (93.4%)
SLN positive	36 (34.6%)
SLN was only positive lymph node	16 (44.4%)
SLN identification method	
Radiocolloid and blue dye	91 (87.5%)
Blue dye only	13 (12.5%)

Table 3. Comparison of lymph node status of SLNs and non-SLNs (n=97)

	Non-SLN status	
SLN status	Positive	Negative
Positive	20	16
Negative	4	57

False-negative rate, 10%; overall accuracy, 96%; negative predictive value, 93%; positive predictive value, 100%

T4d primary tumor.

As seen in Table 2, the overall SLN identification rate was 93.4% (97 of 104). Of the 97 patients in whom an SLN could be identified, 36 (34.6%) had positive SLNs. In 16 of these patients (44.4%), the SLN was the only positive node. SLNs were identified by both radiocolloid and blue dye in 91 patients (87.5%) and by blue dye alone in 13 patients (12.5%).

The pathological status of the SLNs and non-SLNs is outlined in Table 3.

The SLNs accurately predicted axillary status in 93/97 patients (95.9%). Four patients had false-negative SLN biopsies, a false-negative rate of 10.0% (4/40). Fifty-seven patients had pathologically negative SLN or non-SLN.

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

	T2 (n=59)		T3/T4 (n=38)	
	Non- SLN status			
SLN status	Positive	Negative	Positive	Negative
Positive	7	7	13	9
Negative	2	43	2	14
	SLN identified, 59/61 (97%) False-negative rate, 13%		SLN identified, 38/43 (88%) False-negative rate, 8%	

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

In T2 tumors before NAC, the SLN identification rate was 97% (59 of 61), and 2 patients had false-negative SLN biopsies, or a false-negative rate of 13%. In T3 and T4 tumors, the results were 88.4% (38 of 43) and 8%, respectively (Table 4). The SLN identification rate tended to be higher in patients with a T2 primary tumor before NAC than in those with T3/T4 primary tumor before NAC, but the difference was not statistically significant.

In the SLN biopsy results, there was no significant difference between nodal status prior to NAC.

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

	N0 (n=52)		N1/N2 (n=45)	
	Non- SLN status			
SLN status	Positive	Negative	Positive	Negative
Positive	4	8	16	8
Negative	2	38	2	19
	SLN identified, 52/54 (96%) False-negative rate, 14%		SLN identified, 45/50 (90%) False-negative rate, 7%	

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

	CR (n=50)		PR/SD (n=47)	
	Non- SLN status			
SLN status	Positive	Negative	Positive	Negative
Positive	6	5	14	11
Negative	2	37	2	20
	SLN identified, 50/55 (91%) False-negative rate, 15%		SLN identified, 47/49 (96%) False-negative rate, 7%	

Table 7. Success rate of sentinel node identification according to tumor characteristics

	No. of Attempted	Success Rate (%)	P
Tumor classification			
T2	61	97 %	*N.S.*
T3	35	94 %	
T4	8	63 %	
Clinical nodal status			
Negative	54	96 %	*N.S.*
Positive	50	90 %	
Clinical tumor response			
CR	55	91 %	*N.S.*
PR/SD	49	96 %	
Pathological tumor response			
pCR	23	91%	*N.S.*
pINV	81	94 %	

In the patients with clinically negative lymph nodes (N0) before NAC, the SLN identification rate was 96.3% (52 of 54), and two patients had a false-negative SLN biopsy, a false-negative rate of 14%. In the patients with clinically positive lymph nodes (N1/N2), the results were 90% (45 of 50) and 7%, respectively (Table 5). In the SLN biopsy results, there was no significant difference between nodal status prior to NAC.

For patients with complete tumor response (CR) after NAC, the SLN identification rate was 91.0% (50/55) and two patients had false-negative SLN biopsies, resulting in a false-negative rate of 15%. For patients with partial tumor response (PR) and stable disease (SD), the results were 96.0% (47/49) and 7%, respectively (Table 6). The SLN identification rate tended to be lower, although the difference was not statistically significant, after NAC in patients with CR after NAC as compared to those with PR and SD.

There was no significant difference in the false-negative rate according to the tumor classification before NAC, the clinical lymph node status before NAC, or the tumor responses after NAC.

There was also no significant difference in the success rate of SLN identification according to tumor classifications before NAC, the clinical lymph node status before NAC, the clinical response of the tumor after NAC, or the pathological response of the tumor after NAC, although the success rate tended to be lower in patients with a T4 primary tumor (Table 7).

Discussion

Although the use of SLN biopsy has dramatically increased over the past several years, and some experienced surgeons are performing this procedure without completing axillary dissection, it is unlikely that SLN biopsy will become the generally accepted standard of care in axillary staging until results from ongoing randomized trials

Table 8. Studies of SLN biopsy after NAC

	No. of patients	Stage	Tumor size (cm)	No (%) of successful SLN biopsies	False negative (%)
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 [12]	29	Any T, N0	NS	27 (93.0)	0 (0)
Nason et al.,2000 [13]	15	T2-4, N0	NS	13 (87.0)	3 (33)
Shimazu et al.,2004 [14]	47	II or III	4.5	44 (93.6)	4 (12)
Current study	104	T2-4, any N	4.9	97 (93.0)	4 (10)

demonstrate the equivalence of this procedure with axillary dissection in terms of axillary recurrence and overall survival. At the same time, it is unlikely that the value of sentinel node biopsy following NAC will be established[1]. The main reason for this is that only a small proportion of operable breast cancer patients currently receive NAC, making a randomized trial quite difficult. Another reason is that when the results from the ongoing randomized trials are disclosed, if they are favorable towards the SLN biopsy procedure, the majority of surgeons will extrapolate the applicability of these results to patients who have received NAC. Thus, it is quite possible that demonstrating the feasibility and efficacy of SLN biopsy after NAC will depend on the retrospective data of single-institution experiences.

NAC can reduce tumor size and significantly increase the ability to perform breast-conserving therapy[16-18]. After NAC, axillary downstaging is similarly affected. NAC with anthracycline/cyclophosphamide-containing regimens has been shown to neutralize the involved axillary nodes in about 30% of patients[16]. The addition of taxanes to anthracycline/cyclophosphamide-containing regimens has increased the conversion rate to around 40%[19, 20]. With the number of patients receiving NAC increasing, the question arises as to whether SLN biopsy is an option for these patients. We summarize the studies regarding SLN biopsy after NAC in Table 8, but they are inconclusive[6-14]. Breslin *et al.*[6] reported a study of 51 patients who underwent SLN biopsy after NAC and concluded that SLN biopsy following NAC is accurate. They had an identification rate of 84.3% and a false-negative rate of 12.0%. Nason *et al.*[13] reported a smaller number of patients who hard received NAC, and their identification and false-negative rates were 87.0% and 33.3%, respectively. They concluded that SLN biopsy resulted in an unacceptably high false-positive rate. However, in these small series, even 1 or 2 patients with false-negative SLNs can greatly affect the conclusions in a different direction. We report here a study of 104 patients who received NAC and had an identification rate of 93.4% and false-negative rate of 10.0%. We conclude in our study that SLN biopsy after NAC is accurate and feasible even for large tumors and patients with positive axillary nodal status before NAC without inflammatory breast cancer.

It has been speculated that among patients who have had 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 might then cause an increased false-negative rate for SLN biopsy and a decreased identification rate of SLN biopsy. However the hypothesis of the present study is that the lymphatic flow around skin lesions is rich and less influenced by the effects of chemotherapy and tumor size than that in the parenchyma surrounding the tumor. The lymph-scintigraphy in this study results before and after NAC demonstrated that the effect of NAC did not at all change the lymphatic flow of the breast.

The results of our study suggest that SLN biopsy after NAC using intradermal injection of radiocolloid is feasible and can accurately predict axillary lymph node status for patients with clinically negative lymph node status following NAC. This procedure could help patients who have had their

axillary lymph node status downstaged from positive to negative and patients with large tumors qualify as appropriate candidates for SLN biopsy.

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

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Original Article

The prevalence of intrinsic subtypes and prognosis in breast cancer patients of different races

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Abstract

A recent report indicated that a high prevalence of basal-like breast tumors (estrogen receptor [ER]-negative, progesterone receptor [PR]-negative, human epidermal growth factor receptor [HER] 2-negative, and cytokeratin 5/6-positive and/or HER1-positive) could contribute to a poor prognosis in African American women with breast cancer. It has been reported that Japanese women with breast cancer have a significantly better survival rate than other races in the USA. These findings suggest that breast cancers in Japanese women have favorable biological characteristics. To clarify this hypothesis, we conducted a cohort study to investigate the prevalence of intrinsic subtypes and prognosis for each subtype in 793 Japanese patients. This study revealed a very low prevalence (only 8%) of basal-like breast tumors with aggressive biological characteristics in Japanese patients. Survival analysis showed a significantly poorer prognosis in patients with basal-like tumors than in those with luminal A tumors (ER- and/or PR-positive, and HER2-negative) with favorable biological characteristics. These findings support the hypothesis that breast cancers in Japanese women have more favorable biological characteristics and a better prognosis than those in other races. In conclusion, the prevalence of basal-like breast tumors could influence the prognosis of breast cancer patients of different races. The prevalence of intrinsic subtypes should be taken into account when analyzing survival data in a multi-racial/international clinical study.

Keywords: Breast cancer; Intrinsic subtype; Triple-negative tumor; Prevalence; Japanese; Prognosis

Introduction

Although breast cancer survival has improved over the past 20 years in some developed countries,[1] significant differences in breast cancer stage, treatments, and mortality rates still exist in the world with regard to race and ethnicity.[2] The causes of survival difference are likely to be multifactorial including socio-economical factors, differences in access to insurance, screening and treatments, and biological differences among breast cancers themselves. These biological differences may reflect genetic influences and differences in lifestyle, nutrition or environmental exposure.

A number of studies have investigated the causative factors leading to racial disparity in breast cancer survival

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between African American (AA) and white American patients in the USA. Possible explanations include aggressive phenotypes of breast tumors,[3–5] such as high-grade and estrogen receptor (ER)-negative (ER−), patient characteristics,[6,7] such as obesity and a higher rate of co-morbidity, inadequate mammmographic screening,[8,9] delay of diagnosis leading to advanced stage,[10,11] and inadequate treatment,[12–14] such as not meeting treatment guidelines in AA women; however, these factors are unable to totally elucidate the disparity. Interestingly, a recent report indicated that a higher prevalence of basal-like breast tumors (ER−, progesterone receptor negative [PR−], human epidermal growth factor receptor 2-negative [HER2−], cytokeratin [CK] 5/6-positive, and/or HER1-positive [HER1+]), which have aggressive biological phenotypes and a poor outcome, and a lower prevalence of luminal A tumors (ER+ and/or PR+, and HER2−), which have an estrogen-responsive phenotype and a favorable outcome, could contribute to a poorer prognosis in young AA women with breast cancer.[15]

In contrast to AA patients, according to the Hawaii Tumor Registry of the Surveillance, Epidemiology, and End Results Program in the USA, Japanese patients with breast cancer have a significantly better survival rate than patients of other races after controlling for age, stage, and ER/PR status. There are no differences, however, in the survival rates of Chinese, Filipino, and Caucasian women.[16] These findings suggest that breast cancers in Japanese women have favorable biological characteristics, such as a lower prevalence of basal-like breast tumors. To clarify this hypothesis, we conducted a retrospective cohort study to investigate the prevalence of intrinsic subtypes of breast tumors and prognosis for each subtype in Japanese breast cancer patients.

Patients and methods

Study patients

The goal of the present study was to estimate the prevalence of breast cancer subtypes in Japanese breast cancer patients, and to examine correlations between clinico-pathologic variables and survival. Clinico-pathologic data of a cohort of consecutive Japanese patients with invasive breast cancer treated between January 2000 and December 2003 were collected from three different institutes, Kawasaki Medical School Hospital, Tohoku University Hospital, and Tohoku Kousai Hospital in Japan. The study procedures were approved by the institutional review board of each hospital.

Based on the histologic records, tumors were classified into two categories: invasive ductal carcinomas not otherwise specified (NOS) and others. The American Joint Committee on Cancer (AJCC, 5th edition) stage and lymph node status were collected from the medical records. Histologic grading was according to the modified Bloom and Richardson method by Elston and Ellis (Nottingham's grading system).[17] Lymph vessel invasion (LVI) was assessed using hematoxylin–eosin-stained glass slides. Vascular channels lined by thin endothelial cells, especially close to the small arteries and veins, were considered as lymph vessels, and tumor emboli were floating in the lumen in LVI-positive cases. Most LVI were seen at the periphery of the invasive tumors.[18] Blood vessel invasion (BVI) was evaluated using elastica Masson stain or immunostaining for CD34. Tumor cell nests surrounded by elastic fibers and the wall of smooth muscle, next to the small arteries (but not mammary ducts with multilayered elastic fibers) were considered as positive.[18]

Immunohistochemical (IHC) subtypes

ER and PR status were determined by IHC performed at each institute. The cutoffs for receptor positivity were 10%. The HER2 status was also determined by IHC at each institute. According to the criteria of the HecepTest, scores 0 and 1 were considered negative, and scores 2 and 3 were considered positive.[19] Triple-negative (ER−, PR−, and HER2−) breast cancer samples were examined by IHC for CK 5/6 and HER1. CK 5/6 and HER1 were considered positive when more than 10% of the tumor cells were labeled. First antibodies and IHC procedures are presented in Table 1.

According to Carey et al.,[15] IHC intrinsic subtypes were defined as follows: luminal A (ER+ and/or PR+, HER2−), luminal B (ER+ and/or PR+, HER2+), basal-like (ER−, PR−, HER2−, CK 5/6-positive, and/or HER1+), HER2+/ER−, and unclassified (negative for all five markers).

Statistical analysis

Differences between breast cancer subtypes with regard to clinico-pathologic characteristics were examined using analysis of variance, χ^2 tests or Fisher's exact test. Survival curves were generated using the Kaplan–Meier method, and the log-rank test was used to compare mean survival across IHC subtypes. StatView statistical software was used to manage and analyze data. Statistical differences were considered significant at $P \leqslant 0.05$.

Results

IHC subtypes and characteristics of patients

Clinico-pathologic data on 793 Japanese patients with invasive breast cancer were collected from three hospitals in Japan. The characteristics of the patients with IHC data, overall and according to IHC subtypes, are presented in Table 2. IHC subtypes differed significantly by age ($P = 0.025$), AJCC stage ($P < 0.001$), histologic grade ($P < 0.001$), LVI ($P = 0.018$), and BVI ($P = 0.026$). Patients with the basal-like subtype were younger than patients with the HER2+/ER− subtype. Patients with basal-like tumors were more likely to be in the more advanced stage, and to have tumors with a higher histologic grade or BVI than patients with luminal A tumors.