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ORIGINAL ARTICLE

Combination chemotherapy with S-1 plus cisplatin for gastric cancer that recurs after adjuvant chemotherapy with S-1: multi-institutional retrospective analysis

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Abstract
Background It is unclear whether S-1 plus cisplatin is effective for patients with recurrent gastric cancer after adjuvant S-1 chemotherapy.
Methods We retrospectively evaluated the efficacy of S-1 plus cisplatin in patients whose gastric cancer recurred after adjuvant S-1 chemotherapy.
Results In the 52 patients evaluated, the median duration of adjuvant S-1 chemotherapy was 8.1 months, and the median recurrence-free interval (RFI) since the last administration of adjuvant S-1 was 6.4 months. Among the 36 patients with measurable lesions, 7 achieved a complete or partial response, and 13 were evaluated as having stable

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disease, for an overall response rate of 19.4% and a disease control rate of 55.6%. For all patients, the median progression-free survival (PFS) was 4.8 months, and the median overall survival (OS) was 12.2 months. Compared with patients with an RFI of <6 months ($n = 25$), patients with an RFI of ≥6 months ($n = 27$) had a significantly higher response rate (5.0 vs. 37.5%, respectively), longer PFS (2.3 vs. 6.2 months, respectively), and longer overall survival (7.3 vs. 16.6 months, respectively). According to a multivariate Cox model including performance status (PS) and reason for discontinuation of adjuvant S-1, an RFI of 6 months was still significantly associated with PFS and OS. *Conclusions* S-1 plus cisplatin is effective for patients with gastric cancer that recurs after adjuvant S-1 chemotherapy, especially for those with an RFI of ≥6 months.

Keywords Adjuvant chemotherapy · Gastric cancer · Recurrence · S-1

Introduction

Gastric cancer is the fourth most common malignancy in the world (988,602 cases in 2008, 7.8% of total malignancy cases) and the second leading cause of cancer death (737,419 deaths, 9.7% of total) [1]. The prognosis of patients with advanced or recurrent gastric cancer remains poor; chemotherapy confers only a minimal survival advantage, with a median survival of approximately 1 year. The most commonly used regimens are combination chemotherapy consisting of a fluoropyrimidine [5-fluorouracil (5-FU) or oral fluoropyrimidine] plus a platinum agent with or without docetaxel or anthracyclines [2–6].

S-1 is an oral anticancer drug composed of the 5-FU prodrug tegafur and two 5-FU modulators; it has achieved high response rates in patients with gastric cancer in phase II studies [7, 8]. In the Japan Clinical Oncology Group (JCOG) 9912 trial, which compared S-1, cisplatin plus irinotecan, and 5-FU, S-1 demonstrated non-inferiority compared to 5-FU [9]. In another phase III trial that compared S-1 alone to S-1 plus cisplatin (SPIRITS trial), S-1 plus cisplatin showed a significantly higher response rate (54 vs. 31%), longer progression-free survival (PFS; 6.0 vs. 4.0 months), and longer overall survival (OS; 13 vs. 11 months) [4]. Also, in a large, non-Japanese, phase III trial (the First-Line Advanced Gastric Cancer Study; FLAGS trial), S-1 plus cisplatin was associated with fewer toxic effects and demonstrated non-inferiority compared with 5-FU plus cisplatin by exploratory analysis [6]. Therefore, S-1 plus cisplatin is now considered to be one of the standard regimens for metastatic or recurrent gastric cancer.

In addition, the ACTS-GC trial has demonstrated that S-1 is also effective as adjuvant chemotherapy for Japanese patients who have undergone curative gastrectomy for locally advanced gastric cancer [10]. However, approximately 30% of patients still develop recurrence after curative resection followed by adjuvant S-1 [10]. As few patients who received adjuvant chemotherapy were included in the phase III trials described above [4, 7, 9], it is unclear whether patients who develop recurrence after adjuvant S-1 could achieve efficacy with S-1 plus cisplatin similar to that achieved in patients without adjuvant chemotherapy. To address this issue, we conducted the following multi-institutional retrospective analysis.

Patients and methods

Patients

This retrospective study was designed to evaluate the efficacy of first-line chemotherapy with S-1 plus cisplatin for recurrence in patients with gastric cancer who had undergone curative gastrectomy followed by adjuvant S-1 chemotherapy. Patients with histopathologically proven recurrent gastric adenocarcinoma after gastrectomy and lymph node dissection with no residual tumor were eligible for analysis. Additional eligibility criteria were: (1) previous adjuvant S-1 chemotherapy at a planned standard dose and schedule (80 mg/m^2 for 28 consecutive days followed by a 14-day rest; 42-day cycles to be repeated for 1 year); (2) Eastern Cooperative Oncology Group performance status (ECOG PS) 0–2; (3) adequate bone marrow, hepatic, and renal function to be treated with S-1 plus cisplatin; (4) evaluable lesions according to Response Evaluation Criteria in Solid Tumors (RECIST ver. 1.1); and (5) treated with a standard regimen of S-1 plus cisplatin (S-1 80 mg/m^2 for 21 consecutive days followed by a 14-day rest; cisplatin 60 mg/m^2 intravenous infusion on day 8; 35-day cycles to be repeated) [4]. Written informed consent for treatment was obtained from each patient prior to treatment initiation. The Institutional Review Board of each participating center approved the study.

Evaluation of treatment and statistical analysis

The tumor response was assessed objectively according to RECIST ver. 1.1, and the best overall response was recorded as the antitumor effect for that patient. The disease control rate (DCR) represented the percentage of patients with a complete response (CR), partial response (PR), or stable disease (SD). PFS was measured from the date of initiation of S-1 plus cisplatin to the date of progressive disease or death from any cause. Time to treatment failure

(TTF) was measured from the date of initiation of S-1 plus cisplatin to the date of last administration of S-1. OS was estimated from the date of initiation of S-1 plus cisplatin to the date of death or last follow-up visit, using the Kaplan–Meier method. The interval from the last administration of adjuvant S-1 to recurrence was defined as the recurrence-free interval (RFI).

The Cox proportional hazards model was used to estimate the impact of the RFI on TTF, PFS, and OS, with adjustment for other factors that were shown to be significant with a univariate log-rank test. *P* values for testing differences between proportions and response rates were calculated with χ^2 tests for homogeneity or for trend, or with Fisher's exact test. Results were considered to be statistically significant when the *P* value was <0.05. All reported *P* values are two-sided. In particular, we compared the response rate, DCR, time to progression (TTP), PFS, and OS between patients with RFIs of ≥6 and <6 months, because several clinical trials in the first-line setting set this interval of ≥6 months as an inclusion criterion [5, 9, 11].

Results

Patient characteristics

A total of 406 patients with recurrent gastric cancer after adjuvant S-1 chemotherapy had received chemotherapy at 18 institutions until October 2010. Among them, 57 patients (14.0%) had received S-1 plus cisplatin as first-line chemotherapy for recurrence. After the exclusion of 5 patients (1 patient with a non-evaluable lesion and 4 patients with insufficient data), 52 patients were included in the final

Table 1 Patient characteristics

Characteristic	All ($n = 52$)	RFI <6 months ($n = 25$)	RFI ≥6 months ($n = 27$)	*P* value
Age, years				
Median (range)	61 (32–77)	59 (32–77)	62 (32–77)	
Gender, *n* (%)				
Male	30 (58)	15 (60)	15 (56)	0.75
Female	22 (42)	10 (40)	12 (44)	
ECOG PS at recurrence, *n* (%)				
0	32 (62)	11 (44)	21 (78)	*0.012*
1	20 (38)	14 (56)	6 (22)	
Histological type[a], *n* (%)				
wel or mod	27 (52)	10 (40)	17 (63)	0.1
por or sig	24 (46)	15 (60)	9 (33)	
Other	1 (2)	–	1 (4)	
Pathological stage[a], *n* (%)				
Stage I or II	8 (15)	4 (16)	4 (15)	0.57
Stage IIIA	17 (33)	6 (24)	11 (41)	
Stage IIIB	15 (29)	8 (32)	7 (26)	
Stage IV	12 (23)	7 (28)	5 (19)	
Site of recurrence, *n* (%)				
Peritoneum	21 (40)	7 (28)	14 (52)	0.08
Lymph node	25 (48)	13 (52)	12 (44)	0.59
Liver	14 (27)	10 (40)	4 (15)	*0.041*
Lung	4 (8)	3 (12)	1 (4)	0.262
Bone	6 (12)	1 (4)	5 (19)	0.102
Local	2 (4)	1 (4)	1 (4)	0.96
Number of recurrence sites, *n* (%)				
1	38 (73)	18 (72)	20 (74)	0.87
2 or more	14 (27)	7 (28)	7 (26)	

P values shown in italics indicate significant differences

RFI Recurrence-free interval, *PS* performance status, *ECOG* Eastern Cooperative Oncology Group, *wel* well-differentiated adenocarcinoma, *mod* moderately differentiated adenocarcinoma, *por* poorly differentiated adenocarcinoma, *sig* signet-ring-cell-like carcinoma

[a] According to the Japanese classification

analysis (Table 1). The median duration of adjuvant S-1 chemotherapy was 8.1 months (range 0.7–37.4 months), and the median RFI since the last administration of adjuvant S-1 was 6.4 months (range 0–81.3 months). Thirty of the 52 patients (57.7%) completed the planned duration of adjuvant S-1 therapy. In contrast, 14 patients discontinued S-1 due to disease recurrence, and 8 patients stopped therapy due to toxicity or patient refusal. Other than PS and liver metastasis, characteristics did not differ significantly between patients with an RFI of ≥6 months ($n = 27$) and those with an RFI of <6 months ($n = 25$) (Table 1).

Treatment results and efficacy

The median TTF was 4.1 months (95% confidence interval [CI] 2.5–5.1 months), with a median duration of follow-up of 32 months. Forty-four patients discontinued S-1 plus cisplatin due to disease progression ($n = 40$, 90.9%) or toxicity ($n = 4$, 9.1%). Of the 36 patients with measurable lesions, 7 achieved a CR ($n = 3$) or a PR ($n = 4$), and 13 were evaluated as having SD, for an overall response rate of 19.4% (95% CI 7.0–37.0%) and a DCR of 55.6% (95% CI 38.1–72.1%). The median PFS was 4.8 months (95% CI 3.9–6.2 months), and the median OS of all patients was 12.2 months (95% CI 10.2–16.6 months) (Fig. 1). Of the 44 patients who had discontinued S-1 plus cisplatin, 31 (70.4%) received second-line or third-line chemotherapy, including taxanes ($n = 25$) or irinotecan ($n = 17$).

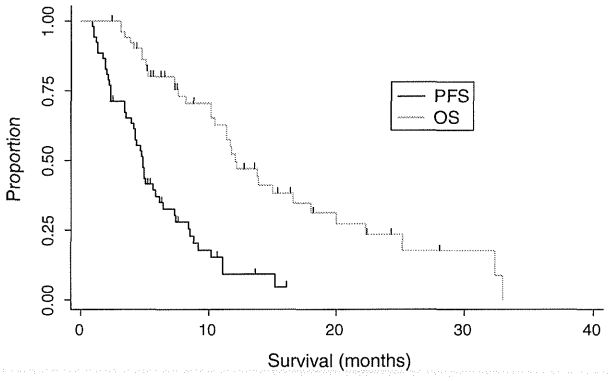

Fig. 1 Progression-free survival (*PFS*) and overall survival (*OS*) in all patients. The median PFS was 4.8 months (95% confidence interval [CI] 3.9–6.2 months), and the median OS was 12.2 months (95% CI 10.2–16.6 months). *PFS* progression-free survival, *OS* overall survival

Significance of the RFI

The response rate was significantly better in patients with an RFI of ≥6 months (37.5%; 95% CI 14–61%) than that in patients with an RFI of <6 months (5.0%; 95% CI 0–15%, $P = 0.014$, Table 2). In addition, compared with patients with an RFI of <6 months, patients with an RFI of ≥6 months had a significantly longer TTF (2.5 vs. 5.1 months, respectively, $P = 0.025$), longer PFS (2.3 vs. 6.2 months, respectively, $P < 0.001$, Fig. 2), and longer OS (7.3 vs. 16.6 months, respectively, $P = 0.003$, Fig. 2). According to a multivariate Cox model including PS and reason for discontinuation of adjuvant S-1, an RFI of 6 months was still significantly associated with PFS (hazard ratio [HR] 0.35, 95% CI 0.16–0.77, $P = 0.009$) and OS (HR 0.21, 95% CI 0.08–0.54, $P = 0.001$), although the association with TTF was not significant (HR 0.55, 95% CI 0.27–1.12, $P = 0.1$). When we divided the patients into two groups based on an RFI of 12 months, no significant difference between the groups was found in response rate, TTP, PFS, or OS.

Discussion

In the ACTS-GC study, adjuvant S-1 chemotherapy significantly improved the survival of patients who had undergone curative gastrectomy for locally advanced gastric cancer [10]. On the other hand, several small studies have suggested that patients with recurrence after adjuvant S-1 were refractory to S-1-containing regimens or had a worse prognosis compared with that of patients without adjuvant chemotherapy [12–14]. Although these reports never precluded the use of adjuvant S-1 chemotherapy, they raised the issue of how to treat recurrent disease after adjuvant S-1.

In the present retrospective study, we evaluated the efficacy of S-1 plus cisplatin in patients whose gastric cancer recurred after adjuvant chemotherapy with S-1. The response rate of 19.4% and PFS of 4.8 months were

Table 2 Objective response rates in patients with measurable lesions

	n	CR	PR	SD	PD	NE	ORR (%)	95% CI (%)
All	36	3	4	13	14	2	18.8	7–32
RFI <6 months	20	0	1	6	13	0	5.0	0–15
RFI ≥6 months	16	3	3	7	1	2	37.5	14–61

CR Complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *NE* not evaluable, *ORR* objective response rate, *CI* confidence interval

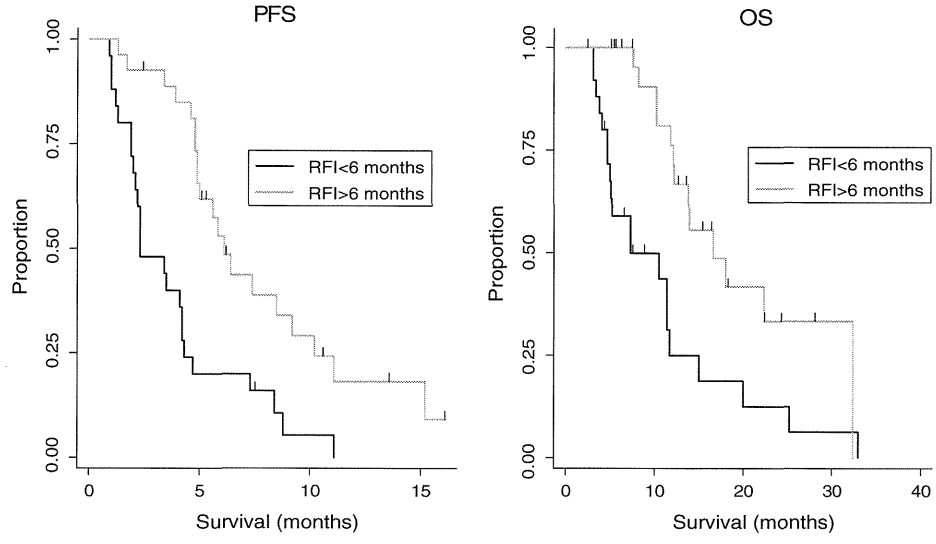

Fig. 2 Progression-free survival (*PFS*) and overall survival (*OS*) according to the length of the recurrence-free interval (*RFI*). Patients with an RFI of ≥6 months had a significantly longer median PFS (6.2 vs. 2.3 months, $P < 0.001$) and OS (16.6 vs. 7.3 months, $P = 0.003$) than patients with an RFI of <6 months. *RFI* recurrence-free interval, PFS progression-free survival, OS overall survival

relatively worse compared with those in the SPIRITS study [4]. However, our results also suggested that patients with an RFI of ≥6 months who received S-1 plus cisplatin had a significantly better response rate, longer PFS, and longer OS compared to patients with an RFI of <6 months. The efficacy of S-1 plus cisplatin for patients with an RFI of ≥6 months in this study was almost compatible with that of patients in the SPIRITS trial in terms of PFS and OS, although these results should be interpreted cautiously due to the heterogeneity of the characteristics of the patients in the two studies. Although no prospective study has evaluated any chemotherapy specifically for patients who have failed adjuvant S-1, Kang and colleagues [15] conducted a phase II study of capecitabine plus cisplatin for 32 patients with gastric cancer that recurred after adjuvant chemotherapy with doxifluridine or 5-FU-containing regimens. They reported a response rate of 28% and a median TTP of 5.8 months, and concluded that capecitabine plus cisplatin was effective as first-line treatment in patients with recurrent gastric cancer after fluoropyrimidine-based adjuvant chemotherapy. In their report, the response rates (21 vs. 39%, $P = 0.427$), TTF (8.3 vs. 5.4 months, $P = 0.072$), and OS (14.1 vs. 9.3 months, $P = 0.075$) tended to be better in patients with an RFI of >6 months ($n = 13$) than in patients with an RFI of ≤6 months ($n = 19$), although the differences did not reach statistical significance [15]. These results were also consistent with those of previous studies in patients with other types of cancer, which suggested the importance of the RFI or treatment-free interval as a predictive marker of responsiveness to similar types of chemotherapy after recurrence [16–18]. Additionally, in the present study, the RFI cut-off value of 6 months was better than that of 12 months for predicting better outcomes and this finding may support the use of the conventional exclusion criteria in clinical trials in the first-line setting, which excluded patients who experienced disease recurrence within 6 months after the last adjuvant chemotherapy [5, 9, 11]. Therefore, selected patients with an RFI of ≥6 months with sufficient organ function may be adequately treated as chemo-naïve patients with standard chemotherapies such as S-1 plus cisplatin.

In contrast to the results for patients with an RFI of ≥6 months, the response rate in patients with an RFI of <6 months in the present study seemed to be worse than that of commonly used second-line chemotherapy regimens such as irinotecan and taxane combinations, which have a reported response rate of approximately 20% for patients with gastric cancer who received prior chemotherapy with fluoropyrimidines alone [18–23]. Based on these results, it may be suggested that the evaluation of chemotherapy regimens other than S-1 plus cisplatin might be warranted for the initial treatment of gastric cancer recurrence after adjuvant S-1. The response rate of 5.0% in our subset of patients with an RFI of <6 months was also lower than that reported previously by Kang et al. for capecitabine plus cisplatin after adjuvant chemotherapy (21%) [15]. The exact reasons for this difference are unknown. One possible reason is that Kang and colleagues did not use the same fluoropyrimidine (capecitabine after doxifluridine or 5-FU), and this choice might have contributed to a higher response in regard to early recurrence, although rechallenge with different types of fluoropyrimidine after the failure of another drug is still controversial in several types of cancer [24–28]. Second, the planned dose intensity of cisplatin as another key drug for gastric cancer was higher in their capecitabine plus cisplatin regimen (60 mg/m^2 every 3 weeks) [15] than that in the S-1 plus cisplatin regimen (60 mg/m^2 every 5 weeks). The efficacy of capecitabine plus cisplatin compared with other

chemotherapy (irinotecan, taxane or irinotecan plus cisplatin) for recurrence after adjuvant S-1 should be evaluated in future clinical trials.

It is important to note the limitations of the present study. First, it was retrospective, and treatment after recurrence was selected by each physician individually. Considering the low proportion of patients who received S-1 plus cisplatin after recurrence (14.0%), the selected population may have been biased toward patients with good performance status (PS) and low tumor burden. Second, toxicity was not evaluated in this study, although the proportion of patients who discontinued S-1 plus cisplatin due to toxicity was low. Third, human epidermal growth factor receptor 2 (HER2) status was not evaluated. Trastuzumab, a humanized monoclonal antibody against HER2, has recently been shown to improve the prognosis of HER2-positive advanced gastric cancer [29], and the HER2 status of all gastric cancer types should be evaluated, even in this setting of recurrent disease. Fourth, the moderate sample size in a single-country study is another limitation; therefore, it would be better to validate the significance of the RFI after adjuvant failure on the PFS in other cohorts as well.

In conclusion, this is the first report to have evaluated the efficacy of chemotherapy with S-1 plus cisplatin in patients with gastric cancer that recurred after adjuvant chemotherapy with S-1. S-1 plus cisplatin was effective in such patients, especially in those with an RFI of $\geq$6 months. Further well-defined, prospective trials in this important patient population are required to identify optimal treatment regimens.

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Conflict of interest None of the authors have financial or personal conflicts of interest to disclose.

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CLINICAL TRIAL

High expression of ATP-binding cassette transporter ABCC11 in breast tumors is associated with aggressive subtypes and low disease-free survival

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Abstract ATP-binding cassette (ABC) transporters are membrane proteins that efflux various compounds from cells, including chemotherapeutic agents, and are known to affect multidrug resistance. Recent reports disagree on whether ABCC11 is a risk factor for breast tumorigenesis, but its expression in breast cancer is poorly investigated. We hypothesized that both frequency and expression levels of ABC transporters in breast tumors would vary by cancer subtype, and be associated with prognosis. Here, we constructed a tissue microarray breast tumor samples from 281 patients, and analyzed expressions of ABCB1, ABCC1, ABCC11, and ABCG2 immunohistochemically. Breast cancer subtypes were determined by immunohistochemistry of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2). Protein expression was correlated to clinicopathological characteristics, clinical follow-up, and pathological complete response to neoadjuvant chemotherapy. The tissue microarray comprised 191 luminal A (68.0 %), 17 luminal B (6.0 %), 27 HER2 (9.6 %), and 46 triple-negative (16.4 %) samples. ABCC1 and ABCC11 expressions were associated with significantly shorter disease-free survival ($P = 0.027$ and $P = 0.003$, respectively). ABCC1, ABCC11, and ABCG2, but not ABCB1, were expressed significantly more, and more frequently, in aggressive subtypes. Patients with HER2+ and triple-negative tumor subtypes that expressed high levels of ABCC11 had significantly worse disease-free survival ($P = 0.017$ and $P < 0.001$, respectively). We have shown, for the first time, that ABCC1, ABCC11, and ABCG2 are highly expressed in aggressive breast cancer subtypes, and that tumor ABCC11 expression is associated with poor prognosis.

Keywords Breast cancer · ATP-binding cassette transporters · ABCC11 · Tissue microarray · Subtype

Introduction

Breast cancer is a heterogeneous disease [1]. DNA microarray profiling studies on breast cancer have identified distinct subtypes: luminal A, luminal B, human epidermal

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growth factor receptor 2 (HER2)-enriched, and triple-negative (which is sometimes further subdivided into the core-basal and five-negative subtypes) [2]. These subtypes are reportedly associated with differences in resistance to chemotherapy [3–5] and subsequent outcomes [6, 7]. Several mechanisms affect how cancer cells become resistant to cytotoxic drugs, which include efflux of the drug compound from cancer cells, and others such as mutation, overexpression of the drug's targets, and drug inactivation [8].

The ATP-binding cassette (ABC) transporters are transmembrane proteins that use ATP to transport various molecules across extra- and intra-cellular membranes. This function is thought to have evolved as a xenobiotic protective mechanism [9]. Of the 49 human ABC transporters so far identified (which have been classified into seven subfamilies), ABCA2, ABCB1, ABCC1–6, ABCC11, and ABCG2 have been associated with chemoresistance in breast cancer [8]. Unfortunately, all clinical trials that have targeted ABC transporters failed to improve outcomes [10]. One explanation for this is that they all targeted ABCB1 [also known as MDR1, permeability glycoprotein 1 (P-glycoprotein or Pgp), and cluster of differentiation 243 (CD243)]. This led us to hypothesize that other ABC transporters may be more important for drug resistance.

ABCC11 is a member of the ABCC1 (also known as MDR-associated protein) sub-family. A single nucleotide polymorphism (SNP) in the *ABCC11* gene was shown to be responsible for "wet earwax" in humans [11]. Reports as to whether *ABCC11* is a risk factor for breast tumorigenesis conflict; although this gene was originally shown to be a risk factor for development of breast cancer among Japanese women [12], it is reportedly not the case in Caucasian women [13, 14]. There has been no investigation of ABCC11 protein expression levels in breast tumors or their association with cancer subtype and prognosis. We hypothesized that both frequency and expression levels of ABC transporters (ABCB1, ABCC1, ABCC11, and ABCG2) in breast tumors would differ by cancer subtype and be associated with prognosis. Here, utilizing a tissue microarray newly constructed from 281 breast cancer samples, we analyzed the expression of these transporters in light of breast cancer subtype and prognosis, as well as investigating the effects of neoadjuvant chemotherapy.

Methods

Tissue sources and clinical characteristics

Tissues for this study were obtained from 281 patients treated in Yokohama City Medical Center, Japan, between 2006 and 2008, involving all stages of breast cancer. This study was approved by the Institutional Review Board of Yokohama City University, Kanagawa, Japan, and the patients gave their informed consent before their inclusion in the study. Core biopsy samples taken prior to treatment were obtained from 50 patients who received neoadjuvant chemotherapy (35 patients received anthracycline followed by taxane; 14 received anthracycline alone; and one received taxane alone). One hundred and eight patients received adjuvant chemotherapy after surgery (45 received anthracycline followed by taxane; 38 received anthracycline alone; 15 received taxane alone; and 10 received other regimens) and 208 patients received adjuvant hormonal therapy (tamoxifen and luteinizing hormone-releasing hormone-agonist for 61 premenopausal patients; tamoxifen or aromatase inhibitor for 147 postmenopausal patients). None of the tissues described here was obtained after any treatment. All the patients were followed up at least every 3 months after surgery. The mean observation period was 49 months (range: 28–60 months). The clinical characteristics are presented in Table 1.

Table 1 Patients' characteristics

	N	%
Age		
<65	197	70.1
65≦	80	28.5
	4	1.4
Menstruation states		
Pre menopause	87	31.0
Post menopause	154	54.8
NA	40	14.2
Estrogen receptor		
Positive	210	74.8
Negative	71	25.2
NA	0	0.0
Progesterone receptor		
Positive	162	42.7
Negative	119	57.3
NA	0	0.0
HER2 overexpression		
Present	44	15.7
Absent	237	84.3
NA	0	0.0
Basal markers		
Basal	34	12.1
Non basal	235	83.4
NA	12	4.5
Subtype		
Luminal A	191	68.0
Luminal B	17	6.0
HER2	27	9.6

Table 1 continued

	N	%
Triple negative	46	16.4
Core basal	26	9.3
Five-negative	20	7.1
Tumor stage		
T1	123	43.8
T2	122	43.4
T3	11	3.9
T4	19	6.8
NA	6	2.1
Node		
N0	150	53.4
N1	83	29.5
N2	23	8.2
N3	11	3.9
NA	14	5.0
Metastases		
M0	259	92.2
M1	6	2.1
NA	16	5.7
TNM stage		
1	106	37.8
2	122	43.4
3	31	11.0
4	6	2.1
NA	16	5.7
Observation time (days)	1458 ± 509[a]	

[a] Expressed as mean ± standard deviation

Tissue microarray

The tissue microarray was constructed by taking 3.0-mm cores from representative areas of surgical specimens from patients using a KIN-2 tissue arrayer (Azumaya, Tokyo, Japan), and re-embedding these cores into a gridded paraffin block. Tissue cores were excluded from the tissue microarray if they fail to adhere to the glass slide, did not include invasive carcinoma, or were a non-interpretable specimen.

Immunohistochemistry

Formalin-fixed paraffin-embedded tissue blocks were sliced into 5-μm sections. The sections were baked at 60 °C, deparaffinized in xylene, and gradually rehydrated in ethanol. Sections were boiled in antigen retrieval solution (Funakoshi, Japan) for 30 min. Activity of endogenous peroxidase was blocked by 20 min of quenching in 0.3 % H_2O_2 and methanol; the sections were then incubated in 5 % rabbit serum for ABCB1 and ABCC1, or goat serum for ABCC11 and ABCG2. Immunohistochemical reactions were performed overnight at 4 °C using monoclonal mouse antibodies against ABCB1 (C219; 1:100; Abcam, UK), monoclonal rat antibodies against ABCC1 (MRPr1; 1:40; Monosan, The Netherlands), polyclonal rabbit antibodies against ABCC11 (1:500) [15], or monoclonal mouse antibodies against ABCG2 (BXP-21; 1:100; Abcam). For the triple-negative subtype, cytokeratin 5/6 (D5/16 B4; Dako, Denmark) and epidermal growth factor receptor (EGFR; Roche Diagnostics K.K, Japan,) were used for subdivision into the core-basal or non-basal (five-negative) subtypes. After washing, the slides were incubated with biotinylated antibodies (15 min, room temperature) and streptavidin-biotinylated peroxidase complex (5 min, room temperature). 3,3′-diaminobenzidine (Dako Japan, Tokyo, Japan) was used as the chromogen. All sections were counterstained with Meyer's hematoxylin.

Evaluation of staining

Staining results were assessed by two pathologists independently, using a 4-point scoring system as shown in Fig. 1: 0 = invasive tumor cells present in the tissue core with no staining; 1 = invasive tumor cells present with weak staining intensity; 2 = invasive tumor cells present with strong staining intensity and <30 % of tumor cells stained or intermediate staining intensity in ≥30 % of tumor cells; and 3 = invasive tumor cells present with strong staining in ≥30 % of tumor cells. To evaluate positivity, both membranous and/or cytoplasmic staining scoring 2 or above was considered positive (high expression). CK5/6 and EGFR were considered positive when cytoplasmic and/or membranous staining of invasive carcinoma cells was observed, regardless of intensity.

Genotyping

Genotyping of ABCC11 by the SmartAmp method was performed as previously reported [12].

Statistical analysis

Statistical analysis used SPSS 19.0 for Windows software (SPSS Inc., Chicago, IL). Correlations among the clinicopathologic parameters and each transporter were evaluated by the Pearson χ^2 test, the Fisher exact test, and the Mann–Whitney test. Tukey-type multiple comparison analyses with the χ^2 test and Mantel test were carried out to compare expression of each transporter among the subtypes. Patient outcomes were assessed by disease-free survival. Survival distributions were estimated by the Kaplan–Meier method; differences were compared using the log-rank test. The multivariate Cox proportional hazard regression method

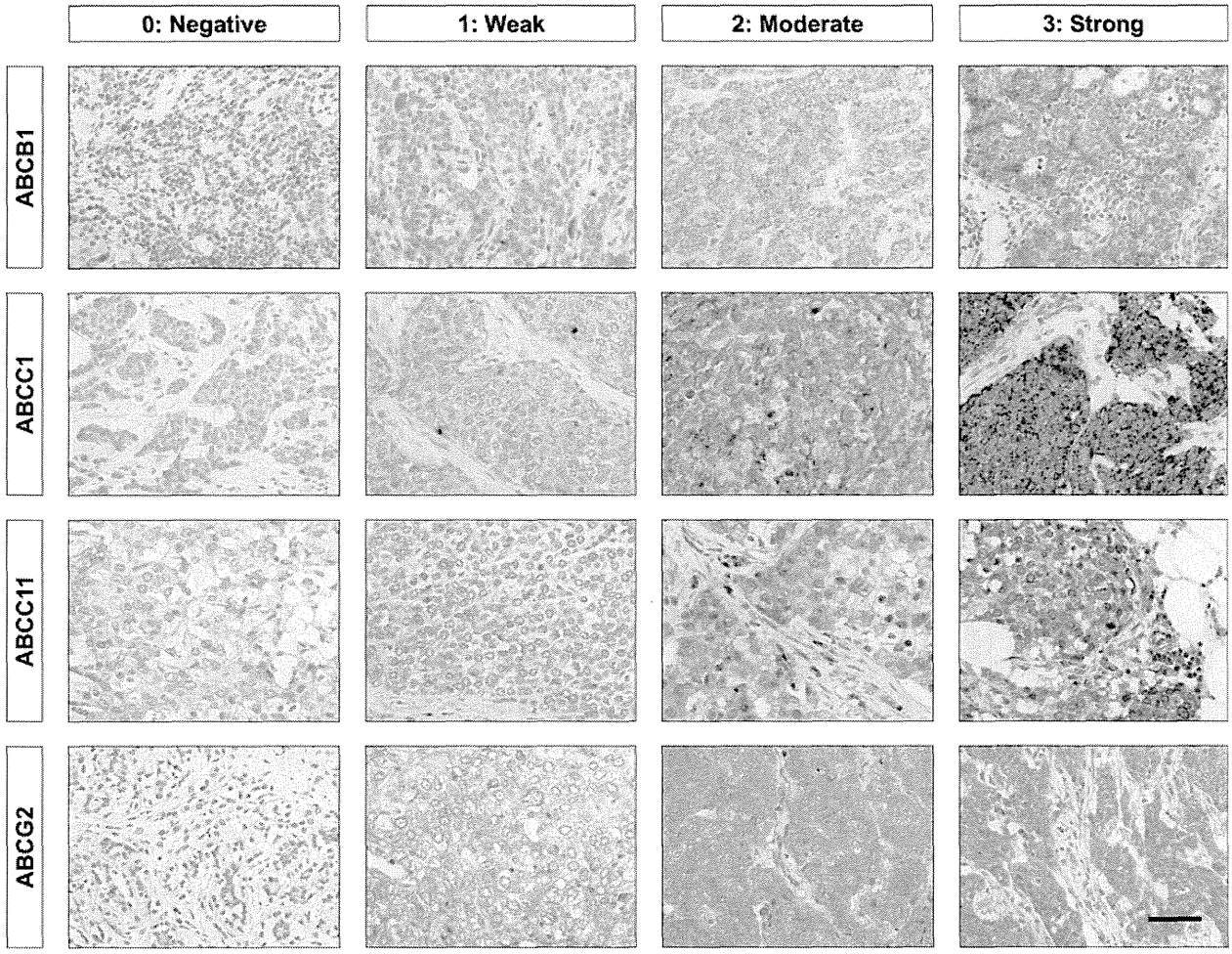


Fig. 1 4-Point scoring system for ABCB1, ABCC1, ABCC11, and ABCG2 protein expression. Our tissue microarray contained 281 breast tumor tissues, and was stained with antibodies against ABCB1 (1:100), ABCC1 (1:40), ABCC11 (1:500), and ABCG2 (1:100). Stain intensity was graded as negative (0), weak (1), moderate (2), or strong (3). Representative images are shown under high magnification. *Scale bar*: 50 μm

was used to determine the independent prognostic value. $P < 0.05$ was considered statistically significant.

Results

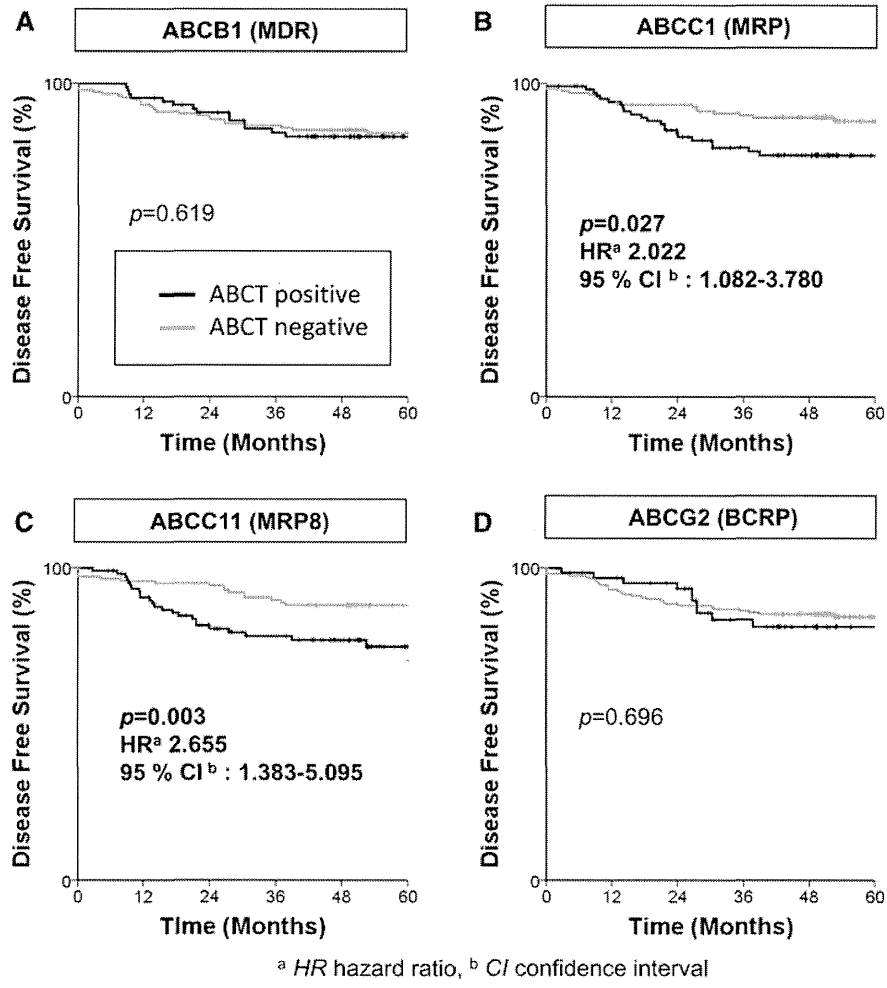
Characteristics of samples used for the tissue microarray

Subtypes of the 281 samples on the tissue microarray were determined using immunohistochemistry for the estrogen receptor (ER), progesterone receptor (PgR), and HER2, as previously reported [5, 16]. Patients' and tumor characteristics used for the tissue microarray are summarized in Table 1. The numbers of cases of the respective subtypes were: luminal A (ER+ and HER2−): 191 (68.0 %); luminal B (ER+ and HER2+): 17 (6.0 %); HER2 (ER− and HER2+): 27 (9.6 %); and triple-negative (ER− and HER2−): 46 (16.4 %). Triple-negative tumors were further sub-divided into two groups, core-basal (CK5/6+ and/or EGFR+) and five-negative (CK5/6− and EGFR−). The core-basal subtype constituted 56.5 % (26/46) of triple-negative tumors.

Associations between ABC transporter expression and clinical features of the tumors are shown in Table 2. ABCB1 was detected in 32.4 % (91/277) of the tumors, ABCC1 in 39.1 % (110/279), ABCC11 in 40.2 % (113/259), and ABCG2 in 24.2 % (68/278). There was no association between ABCB1 expression and any clinical features. ABCG2 was more frequently highly expressed in young premenopausal patients. High expressions of ABCC1 and ABCG2 were significantly more frequent in ER− tumors than in ER + ones ($P = 0.001$ and $P = 0.006$, respectively). There was no association between HER2 expression and ABC transporter expression.

Table 2 The expression of ABC transporters and clinical features

	ABCB1			*p* Value	ABCC1			*p* Value	ABCC11			*p* Value	ABCG2			*p* Value
	Negative	Positive	NA		Negative	Positive	NA		Negative	Positive	NA		Negative	Positive	NA	
N (%)	186 (66.2 %)	91 (32.4 %)	4 (1.4 %)		169 (60.2 %)	110 (39.1 %)	2 (0.7 %)		146 (52.0 %)	113 (40.2 %)	22 (7.8 %)		210 (74.7 %)	68 (24.2 %)	3 (1.1 %)	
Age																
<65	125 (63.5 %)	68 (34.5 %)	4 (2 %)	0.14	119 (60.4 %)	76 (38.6 %)	2 (1 %)	0.54	97 (49.2 %)	83 (42.1 %)	17 (8.7 %)	0.13	138 (70.0 %)	56 (28.4 %)	3 (1.6 %)	<0.01
65≦	58 (72.5 %)	22 (27.5 %)	0		49 (61.2 %)	31 (38.8 %)	0		47 (58.8 %)	28 (35.0 %)	5 (6.2 %)		69 (86.3 %)	11 (13.7 %)	0	
Menstruation status																
Pre menopause	55 (63.2 %)	29 (33.3 %)	3 (3.5 %)	0.40	51 (58.6 %)	36 (41.4 %)	0	0.32	41 (47.2 %)	37 (42.5 %)	9 (10.3 %)	0.35	54 (62.1 %)	31 (35.6 %)	2 (2.3 %)	<0.01
Post menopause	104 (67.5 %)	49 (31.8 %)	1 (0.7 %)		95 (61.7 %)	57 (37.0 %)			81 (52.6 %)	63 (40.9 %)	10 (6.5 %)		81 (52.6 %)	63 (40.9 %)	10 (6.5 %)	
Estrogen receptor																
Negative	50 (70.4 %)	21 (29.6 %)	0	0.61	30 (42.3 %)	39 (54.9 %)	2 (2.8 %)	<0.01	37 (52.1 %	27 (38.0 %)	7 (9.9 %)	0.65	43 (60.6 %)	27 (38.0 %)	1 (1.4 %)	<0.01
positive	135 (64.5 %)	70 (33.5 %)	4 (2.0 %)		139 (66.5 %)	70 (33.5 %)	0		108 (51.7 %)	86 (41.1 %)	15 (7.2 %)		166 (79.4 %)	41 (19.6 %)	2 (1.0 %)	
Progesterone receptor																
Negative	78 (65.5 %)	38 (31.8 %)	3 (2.7 %)	0.78	63 (52.9 %)	54 (45.4 %)	2 (1.7 %)	0.06	57 (47.9 %)	49 (41.2 %)	13 (10.9 %)	0.55	81 (68.0 %)	35 (29.4 %)	3 (2.6 %)	0.15
Positive	107 (66.5 %)	53 (32.9 %)	1 (0.6 %)		106 (65.8 %)	55 (34.2 %)	0		88 (54.7 %)	64 (40.0 %)	9 (5.3 %)		128 (79.5 %)	33 (20.5 %)	0	
HER2 expression																
Absent	155 (66.0 %)	77 (32.8 %)	3 (1.2 %)	1.00	146 (62.1 %)	88 (37.4 %)	1 (0.5 %)	0.18	123 (52.4 %)	97 (41.2 %)	15 (6.4 %)	1.00	179 (76.1 %)	55 (23.4 %)	1 (0.5 %)	0.33
Present	179 (76.1 %)	55 (23.4 %)	1 (0.5 %)		22 (50.0 %)	21 (47.7 %)	1 (2.3 %)		21 (47.7 %)	16 (36.4 %)	7 (15.9 %)		29 (65.9 %)	13 (29.5 %)	2 (4.6 %)	



[a] *HR* hazard ratio, [b] *CI* confidence interval

Fig. 2 Kaplan–Meier disease-free survival curves according to expression of ABCB1 (**a**), ABCC1 (**b**), ABCC11 (**c**), and ABCG2 (**d**). The *thick bold line* indicates positivity; and the *light gray line* indicates negativity, for the respective transporters. Only the ABCC1+ and ABCC11+ groups showed significantly improved survival ($P = 0.027$ and $P = 0.003$, respectively)

Expression of ABCC1 and ABCC11 is associated with poor patient survival

We compared expression of each transporter and patient disease-free survival (Fig. 2). In the entire study group, patients with ABCC1+ or ABCC11+ tumors had significantly shorter disease-free survival compared to patients with corresponding ABCC1− or ABCC11− tumors ($P = 0.027$ or $P = 0.003$, respectively).

ABC transporters are more frequently highly expressed in aggressive subtypes of breast cancer

Because breast cancer subtypes are associated with different clinical behaviors [2], we further analyzed clinical outcomes according to cancer subtype and ABC transporter expression. Expression of each transporter according to breast cancer subtype is shown in Fig. 3. The percentage of patients whose tumors expressed ABCB1 did not differ among the subtypes. ABCC1 and ABCG2 were more frequently highly expressed in triple-negative subtype, especially in the core-basal subtype, compared with the luminal A subtype, whereas highly expressed ABCC11 was more common in HER2-enriched, core-basal, and luminal A subtypes. Although core-basal tumors tended to express ABC transporters more often than five-negative tumors did, only ABCC11 showed significantly more frequent high expression in the core-basal subtype. Semi-quantification of ABC transporters expression is shown in Fig. 3b. ABCC1, ABCC11, and ABCG2 were more highly expressed in HER2-enriched and/or the core-basal subtypes, which is consistent with frequency data shown in Fig. 3a.

Patients whose tumors expressed high levels of ABCC11 tended towards decreased pathological complete responses to neoadjuvant chemotherapy

We next investigated whether there was any association between the "wet earwax" genotypes and ABCC11 expression. Figure 4a and b show the relationship between *ABCC11* genotypes and ABCC11 expression in breast

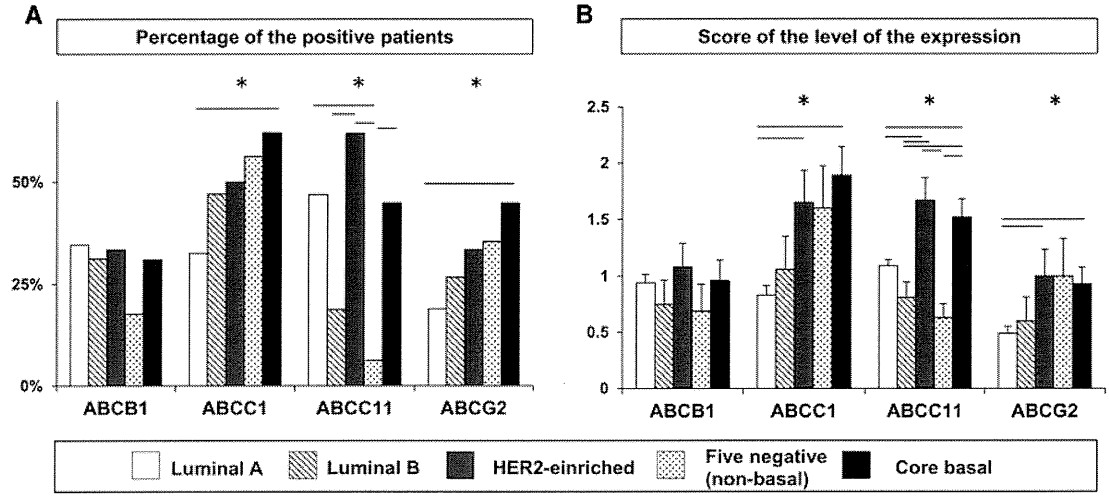


Fig. 3 Frequency (**a**) and intensity (**b**) of high ABC transporter expression classified by subtype, including luminal A (*open columns*), luminal B (*hatched columns*), HER2-enriched (*gray columns*), five-negative (*dotted columns*), and core-basal (*filled columns*). **a** Percentage of patients who showed high expression of each transporter. **b** Semi-quantification of expression level of each transporter, using a 4-point scoring system

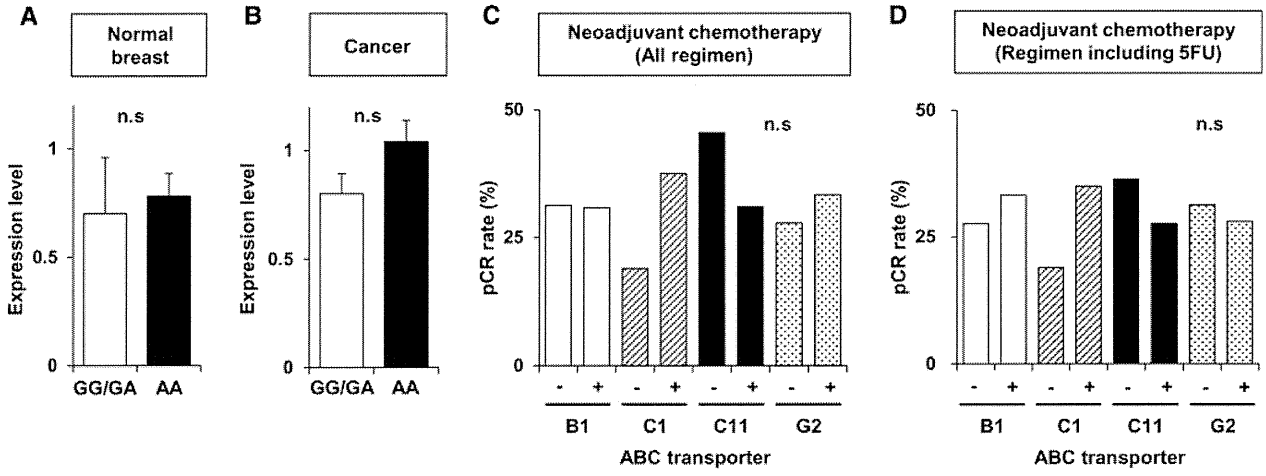


Fig. 4 Semi-quantification of ABCC11 expression levels in normal breast tissue (**a**) and cancer tissue (**b**) in patients carrying 538G/G, 538G/A (white open column, GG/GA, wet earwax phenotype), and 538A/A alleles (*black filled column* AA, dry earwax phenotype). **c**, **d** Pathological complete response ratios to neoadjuvant chemotherapy of all regimens (**c**) and regimens including 5-FU (**d**). *Bars* indicate ABCB1 (*white columns*), ABCC1 (*hatched columns*), ABCC11 (*black columns*), and ABCG2 (*dotted columns*)

cancer tissues. ABCC11 expression did not differ among the wet earwax genotype (538G/G + 538G/A) and the dry earwax genotypes (538A/A), in either normal breast tissues or breast cancer tissues.

As ABCC11 is known to efflux fluoropyrimidines (5-FU) in vitro [17], assessment of responses of ABCC11+ tumors to 5-FU-based regimens could be particularly valuable. Analysis of the association between ABC transporter expression and pathological complete response to neoadjuvant chemotherapy showed no statistically significant differences, regardless of regimen, but patients whose cancers expressed high levels of ABCC11 tended to have decreased pathological complete responses to neoadjuvant chemotherapy (Fig. 4c, d).

ABCC11+ tumors show worse prognoses among aggressive breast cancer subtypes

Because patients with ABCC1+ or ABCC11+ tumors tend to have poor prognoses, we investigated prognosis according to subtype. Patients with ABCC1+ tumors ended to have worse prognoses for luminal A tumors, but not significantly so ($P = 0.096$). Interestingly, patients with ABCC11+ tumors had significantly worse prognoses than did patients

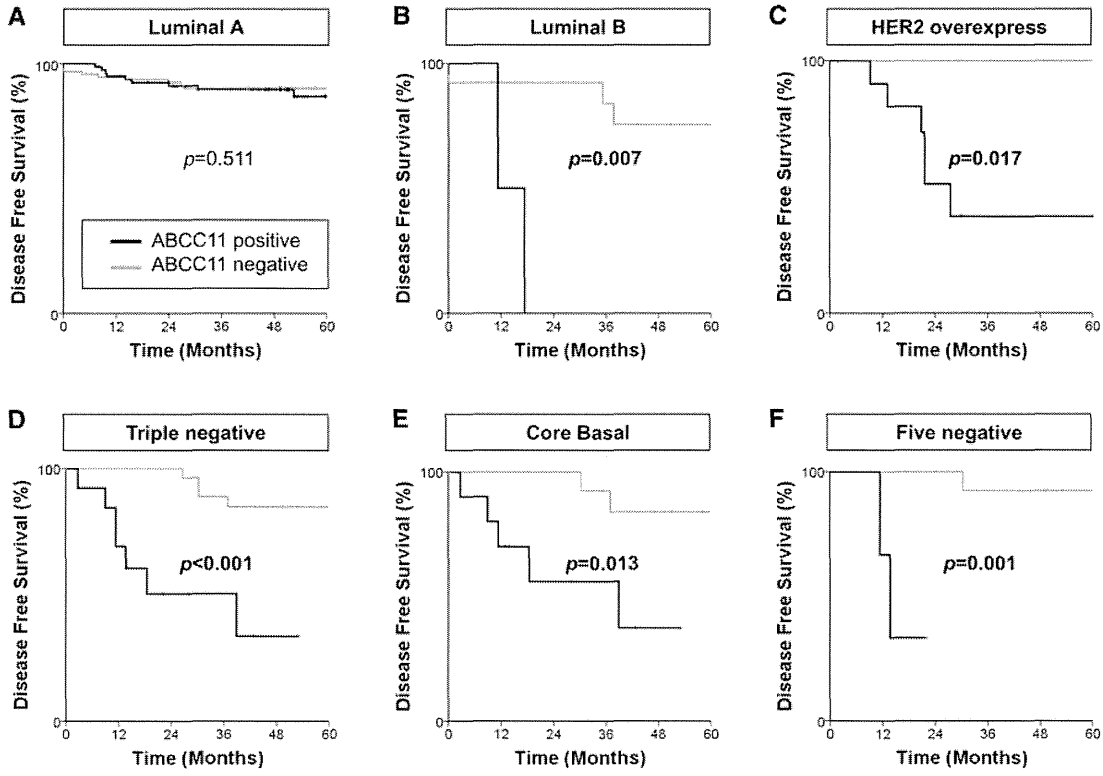


Fig. 5 Kaplan–Meier disease-free survival curve according to the subtype of breast cancer: luminal A (**a**), luminal B(B), HER2-enriched (**c**), triple-negative (**d**), core-basal (**e**), and five-negative (**f**). The *thick bold line* indicates ABCC11+, and the *light gray line* indicates ABCC11−

with ABCC11− tumors, except for the luminal A subtype, which is known to have a better prognosis than the other subtypes (Fig. 5a–f).

Discussion

Different subtypes of breast cancer have different biological behaviors, including responses to systemic and local therapies [3–5] and subsequent clinical outcomes [6, 7]. The two hormone receptor-negative subtypes, triple-negative and HER2-enriched, have poor outcomes compared with the luminal subtypes. Among the triple-negative subtypes, the core-basal subtype, which responds poorly to cytotoxic chemotherapy, has the worst prognosis. Thus, there is a particular need to elucidate drug resistance mechanisms for this subtype.

Expression of ABC transporters is reportedly related to chemoresistance [9]. Some ABC transporters, namely ABCB1, ABCC1, and ABCG2, have been identified as MDR proteins in breast cancer, which contribute to drug resistance via ATP-dependent drug efflux pumps [8]. Because ABCB1 effluxes drugs important for breast cancer—anthracyclines (doxorubicin, epirubicin, and daunomycin) and taxanes (paclitaxel, docetaxel)—ABC transporter inhibitors were the subjects of several widely anticipated clinical trials. Unfortunately, these agents proved disappointing [8, 18]. The vast majority of clinical trials targeting ABC transporters focused on ABCB1 (the most investigated ABC transporter) but data that associates patients' clinicopathological factors with ABCB1 expression tends to conflict [10]. This led us to investigate expression of multiple ABC transporters that are associated with MDR, in the context of different breast cancer subtypes. We felt that this information would be particularly relevant for the triple-negative subtype.

Patient characteristics and our tissue microarray staining data generally agree with previous reports [10, 19, 20]. The proportion of breast cancer subtypes may differ among different races or geographic populations; e.g., prevalence of the luminal A subtype may be higher, and the triple-negative subtype may be lower, in Asian women than in Western women [19]. The demographics of our tissue microarray are consistent with the prevalence among Japanese women. Leonessa et al. [10] reported that the detection rate of ABCB1 and ABCC1 in untreated tumors by immunohistochemistry was 40 % (range: 0–100 %) and 49 % (range: 20–100 %), respectively, with no clear association between ABCB1 and hormone receptors. In

agreement, our results also showed no association between ABCB1 expression and clinical features.

Among ABC transporters, ABCC11 is at relatively early stages of investigation. ABCC11 is lipophilic anion pump that can confer resistance to chemotherapeutic agents such as methotrexate and 5-FU [17]. We previously reported that a SNP in *ABCC11* is associated with the risk of developing breast cancer among Japanese women [12], although the association of *ABCC11* with breast cancer risk is unclear in Caucasian and European women [13, 14]. These reports mentioned host factors that might differ among races and thus modify the impact of this gene on breast cancer risk. *ABCC11* mRNA is reportedly over-expressed in breast tumors and breast cancer cell lines [9, 21, 22], but few studies discuss expression of the ABCC11 protein in human tumors [23]. Although the breast cancer risk conferred by the SNP in *ABCC11* is not within the scope of this study, we did not see significant differences in breast cancer prognosis by SNP genotype in our samples.

Core-basal and HER2-enriched subtypes are associated with poor clinical outcome [5]. In our series, high expressions of ABCC1 and ABCG2 were more common in aggressive subtypes such as core-basal. Strikingly, high expression of ABCC11 was more frequent and intense in both the HER2-enriched and core-basal subtypes, which implies that ABCC11 may promote the aggressive behavior of these subtypes. Indeed, ABCC11 has been shown to export not only drugs but also other factors that affect cancer biology. In agreement, our results show that patients with high tumor expression of ABCC11 have worse outcomes, particularly among the HER2-enriched and core-basal subtypes. This is the first study to show such an association.

Reportedly, ABCC11 expression is related to sensitivity and resistance to chemotherapy [17, 24–26]. In our data, only ABCC11, but not other transporters, tended to correlate with neoadjuvant chemotherapy response. Interestingly, this was true of chemotherapy regimens that both did and did not include 5-FU, which suggests that ABCC11 possesses unidentified supportive functions for drug resistance other than simple drug efflux. For example, we reported that ABCC1 and ABCG2 in breast cancer cells export sphingosine-1-phosphate [27], a bioactive lipid mediator known to affect drug resistance; we cannot exclude the possibility that ABCC11 possesses such a function. In that case, ABCC11 could become a new target in suppressing drug resistance.

Interestingly, it has been suggested that ABCB1 and ABCG2 may affect the role of cancer stem cells in drug resistance [8]. Although we do not currently have data on this relationship, it is intriguing to speculate that the worse prognosis of ABCC11-expressing tumors may be related to cancer stem cells.

Our study is limited in that it is a retrospective analysis of prospectively collected breast tumor samples, and that it shows only association of these transporters with breast cancer prognosis. To evaluate adequately the role of ABCC11 in breast cancer drug resistance, further studies of the mechanism of resistance are needed.

In conclusion, this is the first demonstration that ABCC11 expression in breast cancer is associated with aggressive subtypes and poor disease-free survival.

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Ethical standards This study was approved by the Institutional Review Board of Yokohama City University, Kanagawa, Japan.

Conflicts of interest The authors declare that they have no conflict of interest.

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ORIGINAL REPORT

Randomized, Open-Label, Phase III Study Comparing Irinotecan With Paclitaxel in Patients With Advanced Gastric Cancer Without Severe Peritoneal Metastasis After Failure of Prior Combination Chemotherapy Using Fluoropyrimidine Plus Platinum: WJOG 4007 Trial

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ABSTRACT

Purpose
This phase III study compared treatment with weekly paclitaxel and biweekly irinotecan in patients with advanced gastric cancer refractory to treatment with fluoropyrimidine plus platinum.

Patients and Methods
Patients were randomly assigned to receive either paclitaxel (80 mg/m^2 on days 1, 8, and 15, every 4 weeks) or irinotecan (150 mg/m^2 on days 1 and 15, every 4 weeks). Primary end point was overall survival (OS), and secondary end points were progression-free survival (PFS), response rate, adverse events, and proportion of patients who received third-line chemotherapy.

Results
Of 223 patients, 219 were eligible for analysis. Median OS was 9.5 months in 108 patients allocated to the paclitaxel group and 8.4 months in 111 patients allocated to the irinotecan group (hazard ratio [HR], 1.13; 95% CI, 0.86 to 1.49; P = .38). Median PFS was 3.6 months in the paclitaxel group and 2.3 months in the irinotecan group (HR, 1.14; 95% CI, 0.88 to 1.49; P = .33). Response rate was 20.9% in the paclitaxel group and 13.6% in the irinotecan group (P = .24). Common grade 3 to 4 adverse events were neutropenia (paclitaxel group, 28.7%; irinotecan group, 39.1%), anemia (21.3%; 30.0%), and anorexia (7.4%; 17.3%). Treatment-related deaths occurred in two patients (1.8%) in the irinotecan group. Third-line chemotherapy was administered in 97 patients (89.8%) after paclitaxel treatment and in 80 patients (72.1%) after irinotecan treatment (P = .001).

Conclusion
No statistically significant difference was observed between paclitaxel and irinotecan for OS. Both are reasonable second-line treatment options for advanced gastric cancer.

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INTRODUCTION

The outcomes in patients with unresectable gastric cancer are extremely poor; median survival times of 3 to 5 months have been reported with best supportive care (BSC) alone.[1-3] In randomized studies conducted in the 1990s, first-line chemotherapy for advanced gastric cancer provided survival benefit over BSC alone. After many clinical trials, at present, fluoropyrimidine plus platinum with or without epirubicin or docetaxel is regarded as standard first-line chemotherapy in the treatment of gastric cancer worldwide.[4-9]

Since S-1 was approved for treatment of advanced gastric cancer in Japan, several phase III studies have been conducted, such as the JCOG 9912 (Japan Clinical Oncology Group 9912; fluorouracil *v* S-1 *v* irinotecan plus cisplatin),[10] SPIRITS (S-1 Plus Cisplatin Versus S-1 in a Randomized Controlled Trial in the Treatment for Stomach Cancer; S-1 *v* S-1 plus cisplatin),[9] and GC0301/TOP-002 trials (Gastric Cancer 0301/Topotecin-002; S-1 *v* S-1 plus irinotecan).[11] On the basis of these study results, S-1 plus cisplatin is accepted as standard first-line chemotherapy for advanced gastric cancer

in Japan. Despite no robust evidence of survival benefit, > 70% of participants received second-line chemotherapy in these studies.[9-11]

Many phase II studies of second-line chemotherapy for advanced gastric cancer have been conducted.[12-20] In evaluations of taxanes, administration of both paclitaxel (210 mg/m^2) and docetaxel (60 mg/m^2) on a triweekly schedule resulted in high rates of grade 3 or 4 neutropenia (37% to 88%),[12-14] whereas lower rates of severe neutropenia (3% to 32%) were observed with weekly administration of paclitaxel (80 mg/m^2).[15-18] Regarding efficacy parameters, response rate (RR) and progression-free survival (PFS) were similar for patients on the triweekly and weekly schedules of paclitaxel. Two reports evaluated weekly paclitaxel as second-line chemotherapy, in which median overall survival (OS) was 5 and 6.9 months, respectively.[15,16] In other studies, combination chemotherapy including biweekly administration of irinotecan (150 mg/m^2) as second-line chemotherapy resulted in median OS of 8 to 10 months,[19,20] although toxicity seemed to be more severe than that seen with weekly paclitaxel. Thus, weekly paclitaxel has become the preferable second-line chemotherapy in Japan.

At present, taxanes and irinotecan are two main options for treatment of advanced gastric cancer refractory to fluoropyrimidine plus platinum. However, to our knowledge, no randomized study has directly compared the efficacy of these two treatments. The West Japan Oncology Group (WJOG) conducted a phase III trial (WJOG 4007) comparing paclitaxel with irinotecan in patients with advanced gastric cancer.

PATIENTS AND METHODS

Patients

Eligible patients were age 20 to 75 years with histologically confirmed metastatic or recurrent gastric adenocarcinoma. Other inclusion criteria were Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 to 2; disease progression confirmed by computed tomography (CT), endoscopy, or other imaging technique during or within 1 month after last dose of first-line chemotherapy with fluoropyrimidine plus platinum; no prior chemotherapy with taxanes or irinotecan; and no severe peritoneal metastasis. Severe peritoneal metastasis was defined as ileus or subileus suggested on barium enema examination and moderate to severe ascites exceeding the pelvic cavity on spine CT scan caused by peritoneal metastasis. In case of treatment with adjuvant or neoadjuvant chemotherapy consisting of fluoropyrimidine plus platinum, patients with disease progression during treatment or within 6 months after treatment completion were eligible. Adequate bone marrow, hepatic, and renal functions were also required.

Study Design

WJOG 4007 was a prospective, multicenter, randomized, open-label, parallel-group phase III clinical trial conducted at 37 centers in Japan. The protocol was approved by the independent ethics committee or institutional review board of each participating institution. This trial was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent before study entry. The trial was registered with the University Hospital Medical Information Network.

After checking eligibility, patients were randomly assigned at a 1:1 ratio to receive either paclitaxel or irinotecan. Random assignment was carried out centrally at the data center using minimization method with the following adjustment factors: institution, ECOG PS (0 to 1 *v* 2), and measurable lesions (presence *v* absence). Neither investigators nor patients were blinded to the allocated treatment.

Treatment

Paclitaxel (80 mg/m^2) was administered intravenously on days 1, 8, and 15, every 4 weeks. Patients were premedicated with histamine receptor-1 and -2 blockers and dexamethasone for prophylaxis of allergic reactions 30 minutes before paclitaxel administration. Irinotecan (150 mg/m^2) was administered intravenously on days 1 and 15, every 4 weeks. Dose reduction and/or cycle delays were permitted according to predefined toxicity criteria. Treatment continued until disease progression, occurrence of unacceptable serious toxicity, or patient refusal of further treatment. Subsequent chemotherapy was not specified.

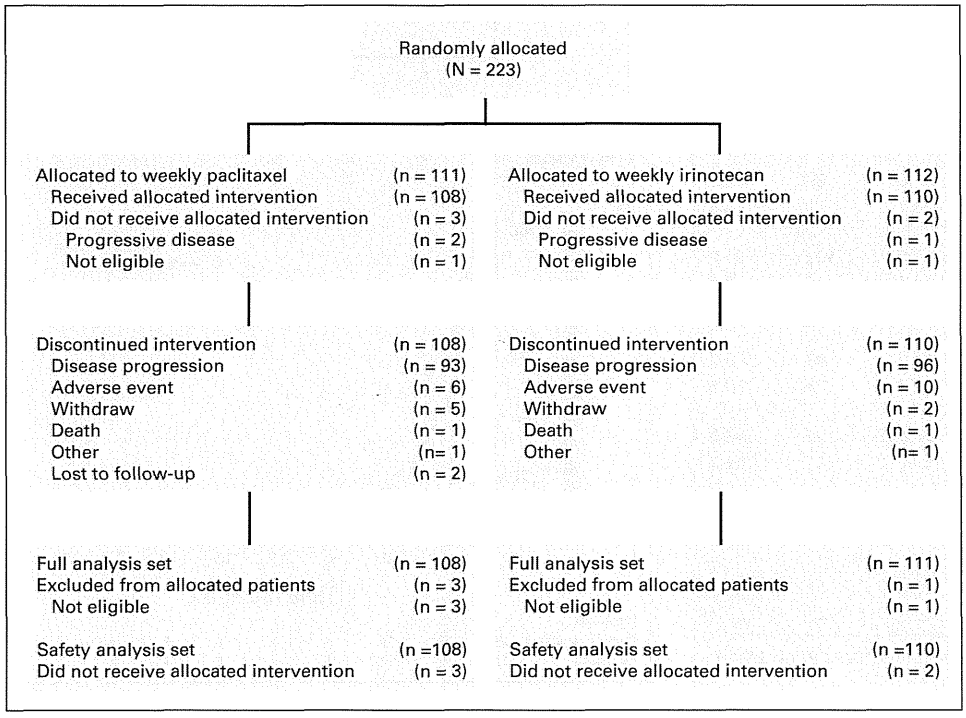

Fig 1. CONSORT diagram.

Assessments

Vital signs, ECOG PS, and laboratory tests were assessed within 7 days before study entry. Physical examinations and hematology and biochemistry tests were conducted during drug administration throughout the treatment course. Tumor assessments using CT scans of the chest, abdomen, and pelvis were performed within 28 days before study entry and repeated every 2 months after random assignment until discontinuation of protocol treatment. RECIST (version 1.0) was used to evaluate treatment responses.[21] Safety assessments were repeated every 2 weeks until initiation of subsequent chemotherapy or 6 weeks after the last protocol treatment. Severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0). The WJOG Data and Safety Monitoring Committee reviewed serious adverse events for trial safety during the protocol treatment. Investigators assessed response, progression, and toxicities in their patients; independent central assessments of response and disease progression were not performed.

Statistical Analysis

The primary end point was OS, defined as time from random assignment to death resulting from any cause. Secondary end points were PFS, defined as time from random assignment to disease progression or death resulting from any cause; RR; toxicity; and proportion of patients who received subsequent chemotherapy.

Previous single-arm studies showed median OS of 5 and 6.9 months in paclitaxel-[15,16] and 8 and 10 months in irinotecan-containing regimen.[19,20] Irinotecan was contraindicated for patients with severe peritoneal metastasis, because its biliary-excreted metabolites caused severe toxicities. In gastric cancer, peritoneal metastasis often developed along with disease progression, and we therefore speculated that subsequent irinotecan after paclitaxel would be more difficult to apply in patients compared with the reverse treatment sequence. On the basis of these previous results and our assumption, this study was designed to detect 50% improvement in median OS from 5 months in the paclitaxel group to 7.5 months in the irinotecan group (hazard ratio [HR], 0.67). Assuming accrual and follow-up periods of 36 and 12 months, respectively, and using a two-sided log-rank test with 5% α and 20% β errors, 220 patients were required for the study. No interim analyses were planned.

A full analysis set (FAS) included all randomly assigned patients who met the eligibility criteria (patients found to be ineligible after random assignment were excluded). The safety analysis set (SAS) included all randomly assigned patients who received $\geq$ one dose of study medication. OS and PFS were analyzed in the FAS and estimated using the Kaplan-Meier method. RR was assessed in patients with $\geq$ one measurable lesion at baseline. Toxicity was analyzed in the SAS.

The primary analysis was planned for 1 year after enrollment of the last patient or approximately 205 events, whichever came first. An independent statistician and data analysis center performed the primary analysis for OS with unstratified log-rank test in the FAS population. All investigators remained blinded to the data until the analysis was completed. Cox proportional hazards models were used to calculate HRs and CIs. Fisher's exact test was used to assess differences in RR, incidence of

Table 1. Baseline Patient Demographic and Clinical Characteristics

Characteristic	Weekly Paclitaxel (n = 108) No.	%	Irinotecan (n = 111) No.	%
Sex				
Male	84	77.7	87	78.4
Female	24	22.2	24	21.6
Age, years				
Median	64.5		65	
Range	37-75		38-75	
ECOG PS				
0 to 1	104	96.3	107	96.4
2	4	3.7	4	3.6
Prior gastrectomy				
Yes	37	34.3	39	35.1
No	71	65.7	72	64.9
Prior chemotherapy				
S-1 plus cisplatin	92	85.2	102	91.9
Capecitabine plus cisplatin	13	12.4	8	7.2
S-1 plus oxaliplatin	3	2.8	1	0.9
Target lesion				
Yes	91	84.3	88	79.3
No	17	15.7	23	20.7
Histology				
Intestinal	54	50.0	54	48.6
Diffuse	54	50.0	57	51.4
Peritoneal metastasis				
Yes	28	25.9	28	25.2
No	80	74.1	83	74.8
No. of metastatic sites				
One	57	52.8	64	57.7
Two or more	51	47.2	47	42.3

Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.

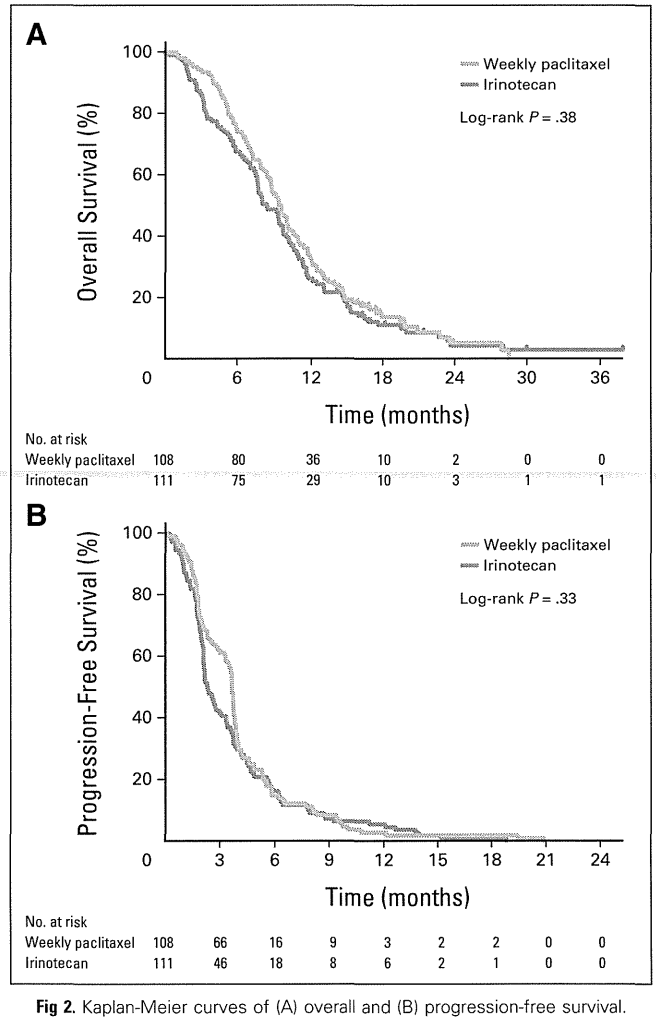

Fig 2. Kaplan-Meier curves of (A) overall and (B) progression-free survival.

