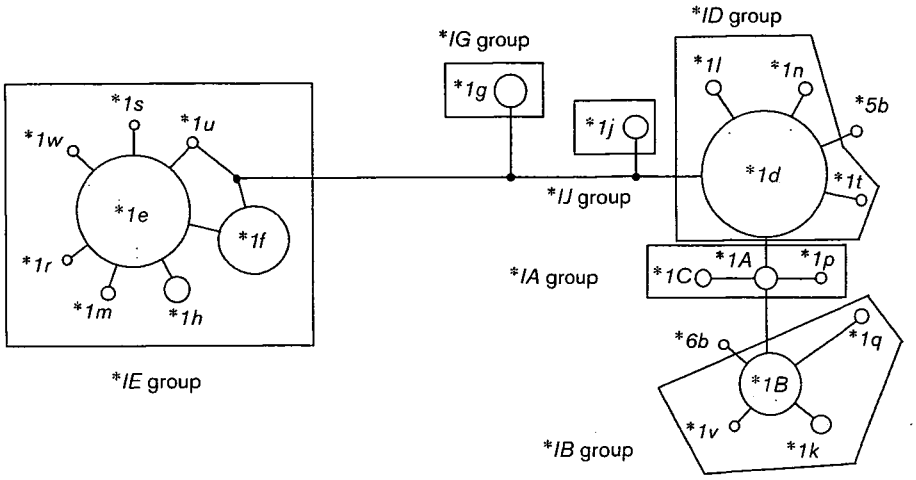
Fig. 3



Network analysis of *CYP2C8* haplotypes. Haplotypes found in at least two patients are shown. The areas of each circle represent the approximate frequency of each haplotype. The **1* subgroups are enclosed by red lines.

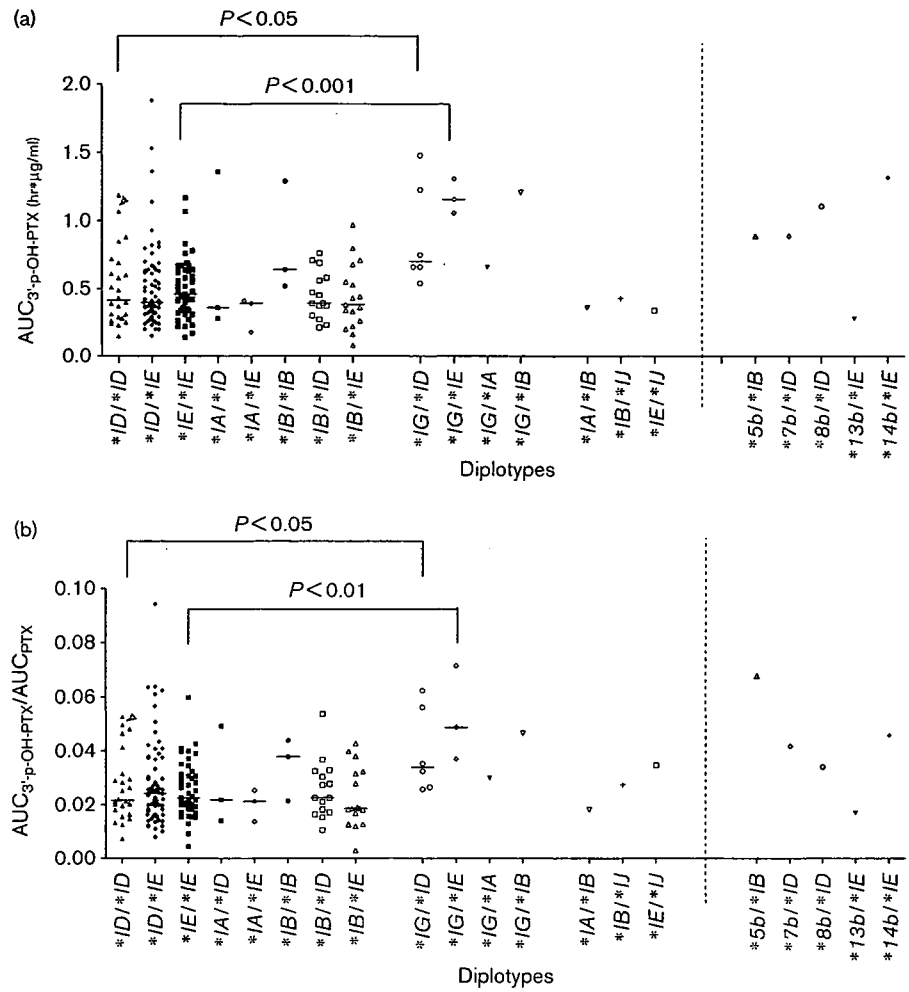
Discussion

All nonsynonymous variations of *CYP2C8* found in Japanese were rare (frequencies ≤ 0.002), and thus we could not apply statistical analysis for their associations with pharmacokinetic parameters of PTX [9]. As shown in Fig. 4b, the AUC ratio of 3′-*p*-OH-PTX/PTX of a patient with heterozygous **5b* haplotype (with 475delA, 159fsX18, no activity) was, however, the third highest (2.8-fold higher than median value) in all 199 patients analyzed. In addition, the patient with heterozygous **7b* (with 556C > T, Arg186X, no activity) had the lowest AUC ratio of 6α-OH-PTX/PTX (approximately one-fifth of the median value) (data not shown). Thus, at least some of the nonsynonymous *CYP2C8* variations described in this paper probably affect the PTX metabolism *in vivo*. These rare variations, however, cannot fully explain the interindividual differences in the CYP2C8 activity. Therefore, we focused on the **1* haplotypes without amino-acid change. The estimated *CYP2C8 *1* haplotypes could be classified into six haplotype groups (**IA*, **IB*, **ID*, **IE*, **IG*, and **IJ*) based on network analysis, and their effects on PTX metabolism were analyzed.

This study revealed that the AUC of 3′-*p*-OH-PTX and AUC ratio of 3′-*p*-OH-PTX/PTX were increased in the **IG*-bearing patients. It must be noted that AUC of 3′-*p*-OH-PTX was considerably increased (2.5-fold). The 3′-*p*-OH-PTX is generated from PTX by CYP3A4 and metabolized into diOH-PTX by CYP2C8. Thus, both CYP2C8 and CYP3A4 activities can influence the AUC of 3′-*p*-OH-PTX. In the previous study [9], we have shown that the *CYP3A4*16B* haplotype harboring 554C > G (Thr185Ser), but not the other haplotypes, increases the AUC ratio of 6α-OH-PTX/PTX and decreases the AUC ratio of 3′-*p*-OH-PTX/PTX with statistical significance. In addition, gender difference was also shown to affect both AUC ratios [9].The association of *CYP2C8*IG* group haplotypes with increased AUC of 3′-*p*-OH-PTX and AUC ratio of 3′-*p*-OH-PTX/PTX, however, could not be explained by the influence of *CYP3A4*16B* (and theoretically null haplotype **6*) or gender difference since the same conclusions were obtained even if patients with *CYP3A4*16B* and **6*, or females were excluded. Moreover, statistical analysis using data only from *CYP3A4*1A/*1A* patients also gave almost the same effects of **IG* on the AUC of 3′-*p*-OH-PTX and the AUC ratio of 3′-*p*-OH-PTX/PTX, suggesting that the effects of *CYP2C8*IG* are independent of the *CYP3A4* genotypes or gender difference. Thus, the increased AUC of 3′-*p*-OH-PTX and AUC ratio of 3′-*p*-OH-PTX/PTX can be attributed to *CYP2C8*IG*, suggesting reduced CYP2C8 activity in patients with **IG*. Moreover, transporters such as P-glycoprotein encoded by the *ABCB1* gene could contribute to the AUCs of PTX and its metabolites [20]. We reported previously that AUC of 3′-*p*-OH-PTX was slightly increased in the patients bearing **2* haplotype in block 2 of *ABCB1* (1236C > T, 2677G > T, and 3435C > T) [9]. When the frequencies of the **2* haplotype were compared between the *CYP2C8*IG/non-*IG* patients and the *non-*IG/non-*IG* patients, however, no statistically significant difference was observed ($P = 0.705$ by χ^2 test).

*CYP2C8*IG* group haplotypes harbors several variations, which are all located in introns. Thus, the mechanism for the increased AUC of 3′-*p*-OH-PTX and AUC ratio of 3′-*p*-OH-PTX/PTX is not caused by an amino-acid change. Among the variations in the **IG* group, IVS3−21T > A

Fig. 4



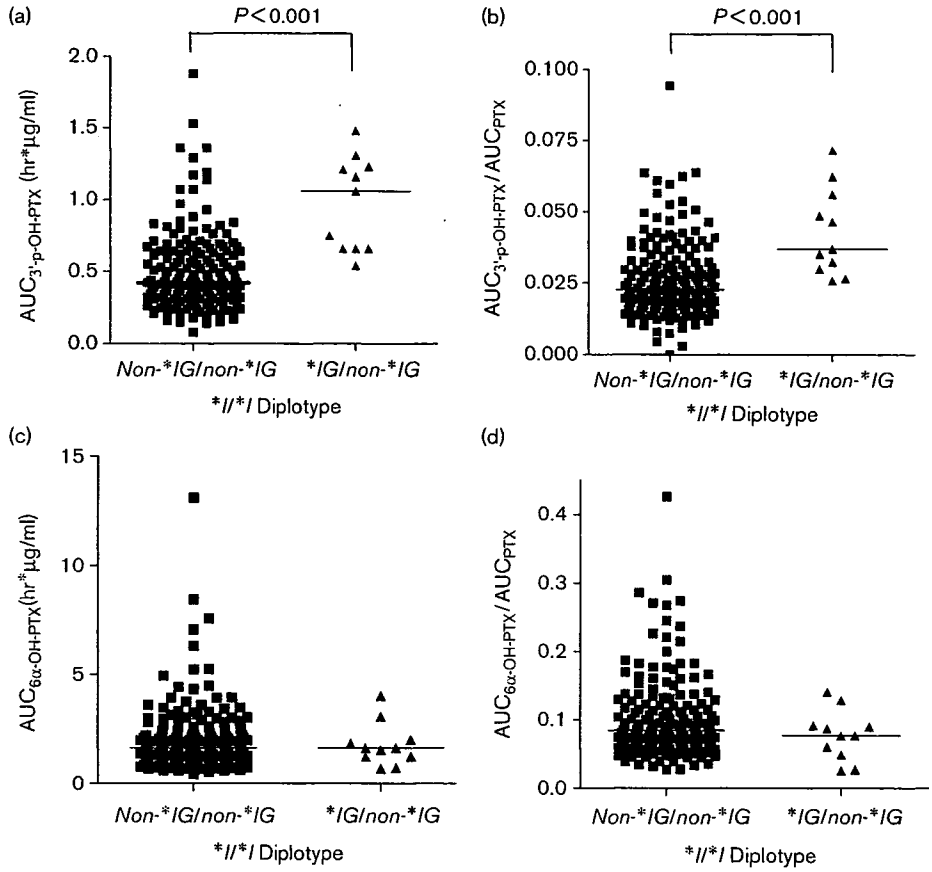
Effects of *CYP2C8* diplotypes on AUC of 3′-*p*-OH-PTX (a), and AUC ratio of 3′-*p*-OH-PTX/PTX (b). All combinations of diplotypes using grouped haplotypes for **1* are shown. Grey arrowheads indicate patients with heterozygous **1t* haplotype. Statistical significance was analyzed by the Mann–Whitney *U*-test to reveal the effects of **1G* group haplotypes. AUC, area under concentration–time curve; PTX, paclitaxel.

and IVS4 + 151G > A were relatively **1G* group specific. Because the patient with **1t* haplotype also had a high AUC of 3′-*p*-OH-PTX and a high AUC ratio of 3′-*p*-OH-PTX/PTX, it is possible that the IVS3 – 21T > A could be a functionally causing variation rather than IVS4 + 151G > A. Because IVS3-21T > A is located in the T-rich (pyrimidine-rich) region upstream of a splice acceptor site and this polypyrimidine tract is important for efficient RNA spliceosome assembly [21], this transversion could reduce the expression level of mature CYP2C8 mRNA, resulting in reduced protein expression levels. We cannot, however, exclude the possibility that other identified/unidentified linked variation could be causative.

We did not observe significant differences in the AUC of 6α-OH-PTX and AUC ratio of 6α-OH-PTX/PTX between the heterozygous **1G* patients and *non-*1G/non-*1G* patients. This is surprising because CYP2C8 is considered to be the major enzyme responsible for 6α-hydroxylation of PTX. Currently, we have no data for explaining this. It is noteworthy that the *CYP3A4*16B* haplotype more clearly affects the increase in AUC ratio of 6α-OH-PTX/PTX than the decrease in AUC ratio of 3′-*p*-OH-PTX/PTX [9]. CYP3A4- and CYP2C8-mediated disappearance processes of 6α-OH-PTX and 3′-*p*-OH-PTX, respectively, might be more influential to their AUCs than their generation from PTX. One alternative (less likely) possibility is that another unidentified enzyme also catalyzes the transformation of PTX into 6α-OH-PTX *in vivo*, and that the effect of reduced CYP2C8 activity is not clearly reflected in the parameters analyzed.

Neither the normalized clearance nor AUC of PTX was significantly influenced by *CYP2C8* diplotypes. The small effect of **1G* on PTX clearance may be partly explained

Fig. 5



Effects of *CYP2C8*IG* group haplotypes on AUC of 3′-*p*-OH-PTX (a), AUC ratio of 3′-*p*-OH-PTX/PTX (b), AUC of 6α-OH-PTX (c), and AUC ratio of 6α-OH-PTX/PTX (d). Statistical significance was analyzed by the Mann–Whitney *U*-test. AUC, area under concentration–time curve; PTX, paclitaxel.

by only small fraction of PTX to be metabolized. In fact, median AUC of 3′-*p*-OH-PTX (0.50 h/mol/l) and 6α-OH-PTX (1.85 h/mol/l) was only 2.3 and 8.5% of that of AUC of PTX (21.67 h/mol/l), respectively.

Recently, Nakajima *et al.* [13] tried to analyze the effects of *CYP2C8* polymorphisms on PTX pharmacokinetics. They genotyped 11 nonsynonymous variations including *CYP2C8*5*, but none were detected from 23 Japanese ovarian cancer patients. Also, we could not apply statistical analysis to the pharmacokinetic parameters for five nonsynonymous variations as described above since the nonsynonymous variations are all rare in Japanese. Rather, **IG* group haplotypes (and possibly **1t*) are probably important for PTX metabolism. The effect of this group haplotypes tagged by IVS3 – 21T > A on pharmacokinetics of other CYP2C8-catalyzing drugs must be clarified in the future.

In conclusion, we determined/inferred a total of 49 haplotypes using the detected variations in the *CYP2C8* gene from 437 Japanese patients. *CYP2C8*IG* group haplotypes, consisting of intronic variations, were found to be associated with significantly increased AUC of the PTX metabolite 3′-*p*-OH-PTX and the AUC ratio of 3′-*p*-OH-PTX/PTX. Thus, *CYP2C8*IG* group haplotypes may influence CYP2C8 activity, although the causative variation is not fully identified.

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Multifaceted psychosocial intervention program for breast cancer patients after first recurrence: Feasibility study

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Abstract

We developed a novel multifaceted psychosocial intervention program which involves screening for psychological distress and comprehensive support including individually tailored psychotherapy and pharmacotherapy provided by mental health professionals. The purpose of the present study was to investigate the feasibility of the intervention program and its preliminary usefulness for reducing clinical psychological distress experienced by patients with recurrent breast cancer. The subjects who participated in the 3 months intervention program completed psychiatric diagnostic interview and several self-reported measures regarding psychological distress, traumatic stress, and quality of life. The assessments were conducted before the intervention (T1), after the intervention (T2), and 3 months after the intervention (T3). A total of 50 patients participated in the study. The rates of participation in and adherence to the intervention program were 85 and 86%, respectively. While the proportion of psychiatric disorders at T2 (11.6%) was not significantly different from that at T1 (22.0%) ($p = 0.15$), the proportion of that at T3 (7.7%) had significantly decreased compared with that at T1 ($p = 0.005$). The novel intervention program is feasible, is a promising strategy for reducing clinically manifested psychological distress and further controlled studies are warranted.

Keywords: psychological distress; recurrence; breast cancer; screening; psychosocial intervention

Introduction

Because cancer is a life-threatening disease, its psychological impact on patients has been an important aspect of clinical oncology. Derogatis *et al.* found that almost half of all cancer patients had been diagnosed as having a psychiatric disorder, and that most of them had an adjustment disorder and/or major depression [1]. Other studies have consistently indicated that adjustment disorders and major depression are common psychiatric disorders among cancer patients [2–4] and are more common in patients with advanced cancer [2,5]. All the previously cited studies have indicated that more than 40% of the breast cancer patients who experienced first recurrence suffer from an adjustment disorder and/or major depression [3,6] and these findings as well as other previous results [7,8] suggest that the impact of cancer recurrence on psychological well-being is serious.

Several studies have indicated that psychological distress can lead to serious and far-reaching negative consequences in patients with advanced cancer, including reducing their quality of life [9] and causing severe suffering [10], a desire for early death, requests for physician-assisted suicide and/or euthanasia [11,12], suicide [13], as well as psychological distress in family members [14]. Maintaining psychological well-being is thus an important issue especially among advanced cancer patients. On the other hand, there are several issues to be overcome in order to reduce psychological distress among cancer patients. For example, under-recognition of the psychological distress, especially depression, experienced by cancer patients is repeatedly reported [15,16]. In addition, a

previous study demonstrated that even when screening for psychological distress was coupled with feedback the results of the screening alone did not seem to contribute to improving the patient's outcome in the oncology setting [17]. Similarly it was reported that the psychosocial intervention by a social worker in combination with screening for psychological distress failed to reduce psychological distress among newly diagnosed breast cancer patients [18]. Lastly recent systematic reviews have suggested that psychotherapy alone does not appear to be sufficiently effective for reducing psychological distress among advanced cancer patients [19,20].

Several recent reports have suggested promising strategies, such as integrated screening programs. These consist of an intervention program that provides pharmacotherapy by oncologists based on antidepressant algorithms [21,22] or psychological intervention delivered by the cancer nurse under the supervision from a consultant psychiatrist, for those patients who have screened positive [23]. In any event, interventions for advanced cancer patients should require a powerful strategy and the previous findings suggest that an integrated program that combines screening for psychological distress with subsequent provision of appropriate treatment and/or intervention seems to be one of the promising strategies.

Based on these findings, we developed a multifaceted psychosocial intervention program which involves screening, psychiatric evaluation, and comprehensive support including individually tailored psychotherapy and pharmacotherapy provided by mental health professionals for cancer patients [24]. The purpose of the present study were: (1) to investigate the feasibility of this novel intervention program and (2) to examine its preliminary usefulness for reducing clinical psychological distress among breast cancer patients who have experienced first recurrence.

Methods

Patients

The subjects were consecutively recruited from outpatient populations of the Oncology-Hematology Division of the National Cancer Center Hospital East (NCCHE) in Japan from January 2001 to January 2002. The eligibility criteria for the study were as follows: (1) histologically, cytologically or clinically proven first recurrence of breast cancer; (2) female, age 20 years or older; (3) informed of recurrent diagnosis; (4) one to six months after the disclosure of recurrence; (5) an estimated life expectancy exceeding six months; (6) follow-up at the Oncology-Hematology Division of the NCCHE; (7) a performance status (PS) of from 0 to 3 according to the Eastern Cooperative Oncology Group criteria. The exclusion criteria were: (1) cognitive impairment; (2) too ill to participate; (3) being treated for any psychiatric disorder by mental health professionals; (4) unable to speak and understand Japanese.

This study was approved by the Institutional Review Board and the Ethics Committee of the National Cancer Center of Japan and was conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from each subject before enrollment into this study.

Study procedures

After written consent was obtained from eligible patients, a psychiatric diagnosis for study outcome (see the outcome measures) was made and sociodemographic data were obtained in a structured interview by a research fellow who was trained for this study ($T1$). The subjects also completed several self-reported questionnaires (see the outcome measures). After that, a multifaceted psychosocial intervention program was provided, including the screening, evaluation interview, and individually tailored intervention. The psychiatrists who provided the intervention were blinded to any subjects' baseline data, including the psychiatric diagnosis at baseline ($T1$). After the intervention, the follow-up assessment was conducted for study outcome at $T2$ and $T3$.

Intervention—multifaceted psychosocial intervention program

The intervention program included multifaceted components (Table 1) and several steps (Figure 1). The program is described in detail elsewhere [25]. Briefly, intervention components consisted of provision of the information material (booklet) for cancer patients, a self-reported brief instrument for screening clinically manifested psychological distress [26], an interview for assessing the patient's physical, psychiatric and psychosocial issues, pharmacotherapy algorithm for major depression [27], and a pharmacotherapy and psychotherapy manual for managing psychological distress for cancer patients. Physical distress items evaluated in the interview were extracted from the MD Anderson Symptom Inventory [28]. Regarding psychiatric diagnosis, the Structured Clinical Interview for DSM- IV (SCID) was included in the interview to evaluate the subjects for major depressive disorder, post traumatic stress disorder (PTSD), and adjustment disorders [29]. The interview form and treatment manual are developed for psychiatrists in residency level. These manuals in Japanese are available from the authors upon request.

As shown in Figure 1, a participant was provided with a booklet and screened for his/her

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Table I. Components of the multifaceted psychosocial intervention

Component	Contents and purpose
Booklet	Medical and psychosocial information for psycho-education
Screening (Distress and Impact Thermometer)	2-item brief self-reported questionnaire for screening clinical psychological distress
Interview form	Semi-structured interview for assessing patient's background, physical symptoms, psychiatric diagnosis, and social support
Pharmacotherapy manual	General attention and practical issues (half-life, metabolism, adverse events, drug-drug interaction, adjuvant use for cancer pain, etc.) for psychotropic medication for cancer patients
Pharmacotherapy algorithm for major depression in advanced cancer patients	Algorithm in consideration of status of advanced cancer such as administration route, estimated prognosis, etc.
Psychotherapy manual	Communication skills and bed side manner for interviewing cancer patients Common psychotherapeutic techniques (psycho-education, crisis intervention, supportive psychotherapy, behavioral therapy, etc.) Special attention for caring for terminally ill cancer patients Defense mechanism frequently observed in cancer patients and management of patients in difficult situations

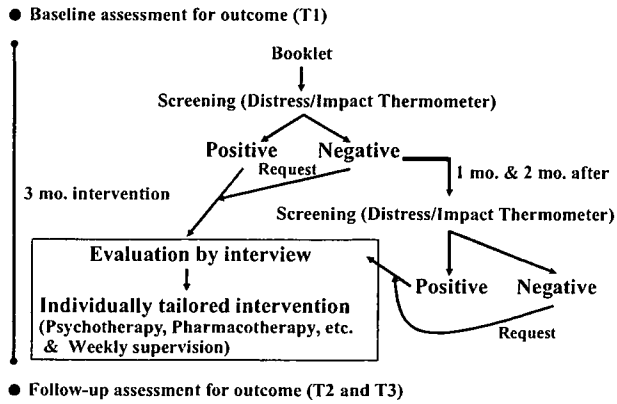

Figure 1. Procedure of the multifaceted psychosocial intervention program and outcome measurements

psychological distress on a monthly basis for a maximum of three times. A patient who screened positive or who screened negative but requested for the following interview was evaluated by the psychiatrist using the interview form. We provided the intervention with the subjects who screened negative but requested the follow-up because a patient's needs can be one of the important factors in determining the morbidity outcome of cancer [30]. After the interview, the patient status was summarized and individually tailored treatment based on the treatment manual was provided by the psychiatrist at residency level. The interview form was designed to be able to clarify patient's uncontrolled physical symptoms, psychosocial issues (e.g. concerns, lack of social support, relationship to medical staff, psychiatric diagnosis), and patient's needs regarding the problems and preferences as to the treatment. Then, just as in actual clinical practice, psychotherapy and/or pharmacotherapy were provided to each patient with flexibility. In most cases, each patient's preference and need for therapy was considered and an appropriate therapy was selected. Regarding psychotherapy, most patients were provided with brief supportive counseling and an additional technique, such as relaxation was also used depending on each patient's situation and preference. The intervention program also included the feedback of information on uncontrolled physical symptoms to patient's oncologists, and sometimes a family meeting was arranged to manage the family's distress. In addition, weekly supervision of treatment procedure was provided to the psychiatric resident by the faculty psychiatrists (T.A., Y.U.). If there was any necessity for continuous psychiatric follow-up at after the end of the intervention, this was provided by the clinical staff of the Psychiatry Division.

Outcome measures

Feasibility

Feasibility of the current program was evaluated in terms of rates of participation and of adherence to the intervention program. The participation rate

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was defined by the participation proportion of the eligible subjects who were informed of the purpose of the present study. We set over 50% of the participation rate as feasible. Adherence to the intervention program was defined by the proportion of the participants who completed the intervention at *T2*. We had set *a priori* that 50% or more of the participation rate and 80% or more of the completion rate would indicate good feasibility. In addition, the total amount of time and number of the interview sessions provided by psychiatrists at residency level and the amount of time of supervision by faculty psychiatrists were recorded. Furthermore, at the end of the study, unsolicited comments regarding the study were obtained from the participants by an interview.

Psychiatric diagnoses

Our primary outcome for this intervention was reduction in the prevalence of diagnosable psychiatric disorders. To evaluate this preliminary usefulness of the intervention program, the prevalence of psychiatric disorders commonly experienced by cancer patients (i.e. adjustment disorders, major depression, and PTSD) among all of the subjects was evaluated using the Structured Clinical Interview for DSM-IV (SCID) by a trained research fellow [29]. These assessment interviews were conducted before the intervention (baseline; *T1*), after the end of the intervention (4 months after the baseline interview; *T2*), and 3 months after the intervention (6 months after the baseline interview; *T3*). The trained research fellow was blinded to any intervention results such as screening and interview findings.

Psychological distress

Psychological distress was evaluated using the Profile of Mood States (POMS) and the Impact of Event Scale—Revised (IES-R). POMS is a 65-item self-rating scale measuring mood disturbance [31]. The POMS is a widely used, reliable measure of emotional distress that has been validated in cancer patients and demonstrated to be reliable for Japanese people [32]. The total mood disturbance (TMD) scale of the POMS, which is the sum of the emotional state subscales, was used. In addition, we measured the impact of being notified of the recurrence of the breast cancer on the patients by the IES-R, which is a 22-item self-rating questionnaire designed to assess the severity of three posttraumatic stress symptoms of intrusion, avoidance, and hyperarousal, and the Japanese version of the IES-R has been shown to be reliable [33,34]. These measures were completed at *T1*, *T2* and *T3*.

Quality of life

Patients' QOL was assessed using the European Organization for the Research and Treatment of Cancer (EORTC) QLQ-C30 and QLQ-BR23. The QLQ-C30 is a 30-item self-report questionnaire covering functional and symptom-related aspects of QOL for cancer patients [35]. The QLQ-BR23 is the breast cancer module, and consists of 23 questions assessing disease symptoms, adverse treatment events, body image, sexuality and future perspective [36]. The validity and reliability of the Japanese version of the EORTC QLQ-C30 and QLQ-BR23 have been confirmed [37,38]. These QOL measures were also completed at *T1*, *T2* and *T3*.

Statistical analysis

To test the preliminary usefulness of the intervention program, the McNemar test was conducted to investigate differences in the proportions of diagnosable psychiatric disorders between *T1* and *T2* and between *T1* and *T3*. As an additional analysis, changes of psychological distress and QOL assessed by POMS, IES-R, QLQ-C30 and QLQ-BR23 among the subjects with clinical psychiatric diagnosis at the baseline were investigated by repeated measures one-way ANOVAs because the important aims of this intervention program involve the early detection of those patients with clinical psychiatric disorders and to provide appropriate psychosocial intervention for them. When a statistical significance was observed, multiple comparisons were conducted using the Dunnett method to clarify which of the psychological distress at *T2* or *T3* differed from the psychological distress at *T1*.

All reported *P*-values are two-tailed. All statistical procedures were conducted using SPSS 12.0J software for Windows (SPSS Inc., 2003).

Results

Characteristics of the participants

During the study entry period, a total of 72 cases of recurrent breast cancer were newly diagnosed; 13 patients were found to be ineligible for enrollment in the study (receiving psychiatric consultation, $n = 4$; no plan of the follow-up, $n = 4$; too ill, $n = 2$; others, $n = 3$) among the remaining 59 eligible patients, 9 patients refused to participate in the study; thus, 50 patients ultimately participated in the study. The characteristics of the subjects are shown in Table 2. Regarding clinical psychiatric disorders, a total of 11 subjects (22%) met any psychiatric diagnoses ('Cases'). Regarding adjustment disorders, four subjects suffered from depressive mood, one from anxious mood, and five from both depressive and anxious moods.

Among the 50 subjects who participated in the study, 43 and 39 subjects completed the *T2* and *T3* follow-up, respectively. The reasons for drop out

Table 2. Baseline characteristics of the patients who participated in the study ($n = 50$)

		No. of patients (%)
Age (mean ± SD, range)		53 ± 10 y, 32–72 y
Education	>= 12 y	40 (80)
Marital status	Married	42 (84)
Household size	Living alone	2 (4)
Children	Presence	43 (86)
Recurrence[a]	Bone	18 (36)
	Lymph node	15 (30)
	Lung	13 (26)
	Liver	10 (20)
	Skin	8 (16)
Current treatment[a]	Chemotherapy	31 (62)
	Hormone	24 (48)
	Radiation	1 (2)
Performance Status[b]	0	40 (80)
	1	8 (16)
	2	2 (4)
Psychiatric diagnoses[c]		
	Adjustment disorders	10 (20)
	Major depression	1 (2)
	PTSD[d]	1 (2)

[a] Multiple choice.
[b] As defined by Eastern Cooperative Oncology Group criteria.
[c] One subject had both adjustment disorder and PTSD.
[d] Posttraumatic stress disorder.

($n = 7$) from the study between $T1$ and $T2$ were as follows: Refusal, $n = 3$; Died, $n = 2$; Too ill, $n = 1$; Transferred to other hospital, $n = 1$. The reasons for drop out ($n = 4$) from the study between $T2$ and $T3$ were as follows: Died, $n = 3$; Refusal, $n = 1$.

Findings regarding the intervention

All of the subjects with psychiatric diagnoses were screened as positive at one of any 3 screening procedures. Three of 11 'Cases' did not receive psychiatric intervention because they said that they did not need any intervention. Among the 'Cases', 2 participants continued to be followed-up by the psychiatry department after the intervention. Among all subjects, a total of 23 subjects received some form of intervention (Psychotherapy, $n = 23$ [Supportive psychotherapy, $n = 23$; progressive muscle relaxation, $n = 5$; crisis intervention, $n = 3$; psycho-education, $n = 2$]; and Pharmacotherapy, $n = 4$ [Benzodiazepines, $n = 3$; antidepressant, $n = 1$]). Contents of intervention provided to the patients differed quite extensively depending on the patient's problems and preferences. For example, some patients received only one interview session because their needs were satisfied or problems were resolved (most of these patients did not have any diagnosable psychiatric disorder) while some others experienced 15 interviews session (see the feasibility). Most of these interventions were arranged and provided for the subjects when they visited the hospital for regular follow-up, medical examination, and anticancer treatment.

Feasibility

The participation rate and adherence of the intervention program were 85% (50/59) and 86% (43/50), respectively. Among the participants who had some form of psychiatric diagnosis at $T1$ ('Cases'), no one dropped out from the study. The number of the interview sessions, the amount of time used for individually tailored intervention by the psychiatry residents, and the amount of time consumed by the supervision were 105 times (mean 5; median 4; range 1–15), 5025 min (approximately 84 h, corresponding with 1.7 h per patient on average), and 2645 min (approximately 44 hours, corresponding with 0.9 hours per patient), respectively. Most of the participants found the intervention program convenient, but a couple of the participants commented that an opportunity of group psychotherapy or a patient group meeting as well as the individual psychotherapy would better satisfy a participant's needs.

Change in prevalence of mental disorders and in psychological distress and QOL

Among the subjects who completed the follow-up, the proportion of psychiatric diagnosis at $T2$ and $T3$ were 11.6% (adjustment disorders, 9.3%; major depression, 2.3%) and 7.7% (adjustment disorders, 7.7%), respectively. While the proportion of $T2$ was not significantly different from that of $T1$ ($p = 0.15$), the proportion of $T3$ was significantly less than that of $T1$ ($p = 0.005$). Given that all of the subjects who dropped out from the study developed clinical psychiatric disorders at $T2$ or $T3$ (worst case scenario), p-values of these comparison were 1.00 ($T2$ vs $T1$) and 0.65 ($T3$ vs $T1$), respectively. Interestingly, the 3 'Cases' at $T1$ who did not receive intervention did not meet any psychiatric diagnosis both at $T2$ and $T3$.

The change of psychological distress evaluated by POMS among the 11 'Cases' was borderline significant ($p = 0.08$) while the total score of the IES-R was significantly reduced ($p = 0.04$) (Table 3). A multiple comparison showed that the total score of the IES-R at $T1$ was significantly reduced at $T3$ ($T1 > T3$, $P = 0.02$). Most other measure including QOL did not change significantly while appetite loss was significantly improved. A multiple comparison showed that appetite loss at $T1$ was significantly reduced at $T3$ ($T1 > T3$, $P = 0.03$).

The measures regarding the psychological distress and QOL among the 39 completed subjects were not statistically significant (data not shown). In addition, those among the 23 patients who had received any kind of treatment were not statistically significant (data not shown).

Table 3. Longitudinal course of psychological distress in patients with clinical psychiatric diagnosis at baseline ('Cases'; $n = 11$)

Psychological Distress	T1 Mean (SD)	T2 Mean (SD)	T3 Mean (SD)	F	p	Multiple comparison
Total mood disturbance (POMS)	45.9 (25.2)	40.8 (27.0)	30.1 (26.4)	2.88	0.08	—
Total score (IES-R)	24.7 (10.6)	19.8 (11.3)	17.3 (10.8)	3.93	0.04	T1 > T3
QLQ C-30						—
Global Health Status	60.6 (22.4)	63.6 (24.5)	56.1 (22.1)	0.79	0.43	—
Physical functioning	80.0 (16.6)	84.2 (14.1)	82.4 (14.7)	0.55	0.59	—
Role functioning	81.8 (21.7)	80.3 (20.8)	83.3 (16.7)	0.10	0.90	—
Emotional functioning	76.5 (12.8)	72.7 (24.5)	82.6 (16.0)	0.83	0.45	—
Cognitive functioning	71.2 (28.0)	75.8 (25.1)	78.8 (21.2)	0.19	0.18	—
Social functioning	83.3 (19.7)	78.8 (21.2)	84.9 (20.4)	0.80	0.46	—
Fatigue	40.4 (14.3)	34.3 (18.9)	38.4 (20.7)	0.70	0.51	—
Nausea and vomiting	21.2 (29.9)	7.6 (17.3)	4.6 (10.8)	1.93	0.17	—
Pain	21.2 (19.8)	18.2 (15.7)	27.3 (27.2)	1.31	0.29	—
Dyspnoea	27.3 (29.1)	18.2 (22.9)	24.2 (26.2)	0.76	0.48	—
Insomnia	12.1 (22.5)	15.2 (17.4)	21.2 (22.5)	0.86	0.44	—
Appetite loss	30.3 (23.4)	12.1 (22.5)	9.1 (21.6)	4.30	0.03	T1 > T3
Constipation	21.2 (22.5)	21.2 (27.0)	6.1 (20.1)	2.34	0.12	—
Diarrhoea	18.2 (22.9)	6.1 (13.5)	18.2 (31.1)	1.16	0.33	—
Financial difficulties	27.3 (32.7)	30.3 (37.9)	24.2 (36.8)	0.41	0.67	—
QLQ BR-23[a]						
Body image	44.7 (26.4)	59.9 (28.3)	59.1 (24.6)	2.29	0.13	—
Future perspective	24.2 (26.2)	39.4 (29.1)	24.2 (26.2)	0.76	0.48	—
Systematic therapy side effects	29.9 (18.9)	27.3 (21.0)	21.7 (17.6)	0.72	0.50	—
Breast symptoms	22.7 (22.1)	22.7 (20.4)	21.2 (16.8)	0.11	0.90	—
Arm symptoms	26.3 (20.7)	24.2 (26.2)	23.2 (24.1)	0.12	0.89	—

[a] Sexual functioning, Sexual enjoyment, and Upset by hair loss were deleted because only a small proportion of the subjects responded to these subscales (Responses, sexual functioning; $n = 2$, sexual enjoyment; $n = 2$, upset by hair loss; $n = 5$).

Discussion

This is the first study to investigate the feasibility of a novel psychosocial intervention program designed to overcome issues of under-recognition and under-treatment of clinically manifested psychological distress often experienced by cancer patients. In addition, for the first time the present study focused on the breast cancer patients after their first recurrence as subjects because of their potentially high and prevalent psychological distress.

In this study, more than 80% of the eligible subjects accepted and participated in the intervention program and also more than 80% of the enrolled participants completed the program. Regarding the participation rate of psychosocial intervention, one previous Japanese study investigating participation in psychosocial group intervention indicated that only 50 (33%) of 151 primary breast cancer patients after surgery actually participated in the group psychotherapy intervention and they suggested the level of participation is lower than in Western countries [39]. The authors suggest several possibilities for the lower level of Japanese psychosocial intervention program and one of them may be due to cross-cultural difference, namely that discussing personal problems with someone outside of the family may bring a deep sense of shame in Asian countries [39]. While we should understand that the difference rate of participation did not result from only the difference of intervention itself (e.g. difference of the subject, study design, etc.), nevertheless these findings suggest that the program, especially the individually tailored style, seems to be highly feasible for recurrent breast cancer patients in Japan. In addition, the adherence rate was also high, which also suggests the high clinical feasibility of the program. These findings suggest that the current modality of the intervention program is feasible and accessible for cancer patients.

The psychiatrists at residency level consumed approximately 84 hours and the faculty psychiatrists did 44 hours for the program, which means that the time taken covered and cared for 50 consecutive breast cancer patients after their recurrence. Concerning appropriate early detection of psychological distress, the brief and repeated screening procedure functioned well as all of the 'Cases' were screened as positive. Although it is not simple to judge cost effectiveness of this kind of intervention, the current findings about the amount of time consumed, including the actual treatment after the brief screening and the supervision for this study, suggest the cost effectiveness of the program.

While the current study design does not allow us to address the precise usefulness of the intervention program for psychological distress experienced by cancer patients, several informative findings were obtained. Among them all, the fact that the proportion of clinical psychiatric diagnoses,

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including mainly depressive disorders, and psychological distress measured by self-reported questionnaires, such as POMS and IES-R can be decreased in the longitudinal course may be promising because our previous findings and the meta-analytical study suggest that psychological distress, especially depression experienced by cancer patients does not improve spontaneously during at least 6 months or one year after cancer diagnosis [4,40,41]. In addition, when target symptoms focused on adjustment disorders and major depression, the previous study failed to show any effectiveness of an antidepressant treatment and there has been no other proven strategy for alleviating these common psychiatric disorders among cancer patients [42]. This multifaceted intervention program may be one promising approach to manage common psychiatric disorders experienced by cancer patients and the current findings warrant a further well-designed study, including randomized controlled clinical trials. On the other hand, most of the quality of life measures were not significantly changed in the present study. Because the current intervention program did not primarily aim at improving the quality of life itself, the findings obtained may not be unexpected. However, when the primary aim is the improvement of the overall quality of life among cancer patients, this multifaceted psychosocial intervention program may not be enough to accomplish the purpose. In that case, additional intervention, such as more powerful and variable intervention components provided by multidisciplinary medical staff and/or additional resources may be needed. It may be important to note that the interventions might help some probable cases or cases at risk other than 'Cases'. Because our previous study indicated that intensive management of a sub-clinical level of anxiety and depression can contribute to the prevention of subsequent clinically manifested psychological distress among advanced cancer patients [43], this procedure may play a role of prevention for developing clinical psychiatric disorders. These findings also warrant further well-designed controlled clinical trials.

The present study has several limitations. First, the small sample size may be one problem and we may have missed some substantive changes (type II error). Second, since the study was conducted in one institution, institutional bias may be another problem and generalizability of the current findings may thus be limited. Third, because the intervention program had a multi-component structure, we cannot know the specific role and/or effectiveness of each part of the intervention. Fourth, because the study lacked a controlled arm, we cannot precisely discern the actual effectiveness of the program from the regression towards the mean, natural course or placebo effect. Finally, because this study focused on patients with recurrent breast cancer, the results may not be applicable to patients with cancer in other foci.

Despite the several limitations of the study, the current findings suggest that this novel intervention program which involves screening for psychological distress and sequentially provided actual psychosocial support may be feasible and one promising intervention strategy for reducing clinical psychological distress experienced by cancer patients and further studies to investigate the effectiveness are warranted.

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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.

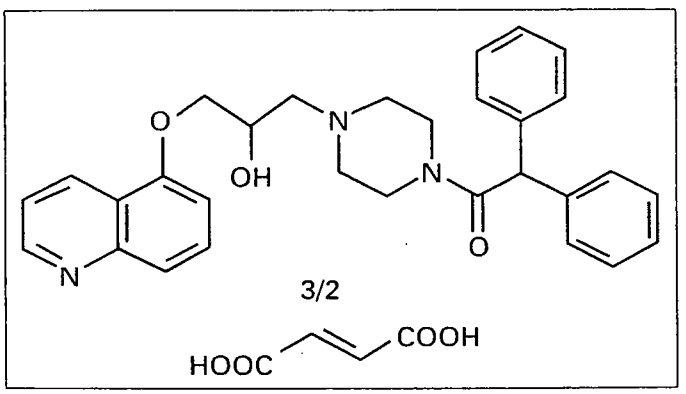

Fig 1. Structure of dofequidar (MS-209).

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.

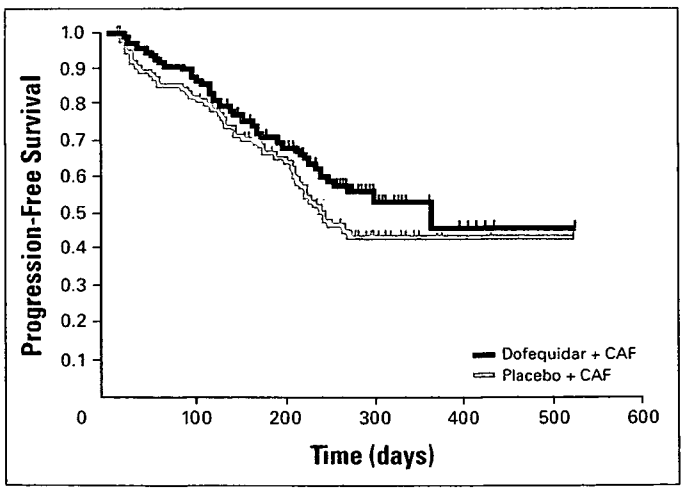

Fig 2. Progression-free survival in patients treated with dofequidar plus cyclophosphamide, doxorubicin, and fluorouracil (CAF) and placebo plus CAF ($P = .145$).

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

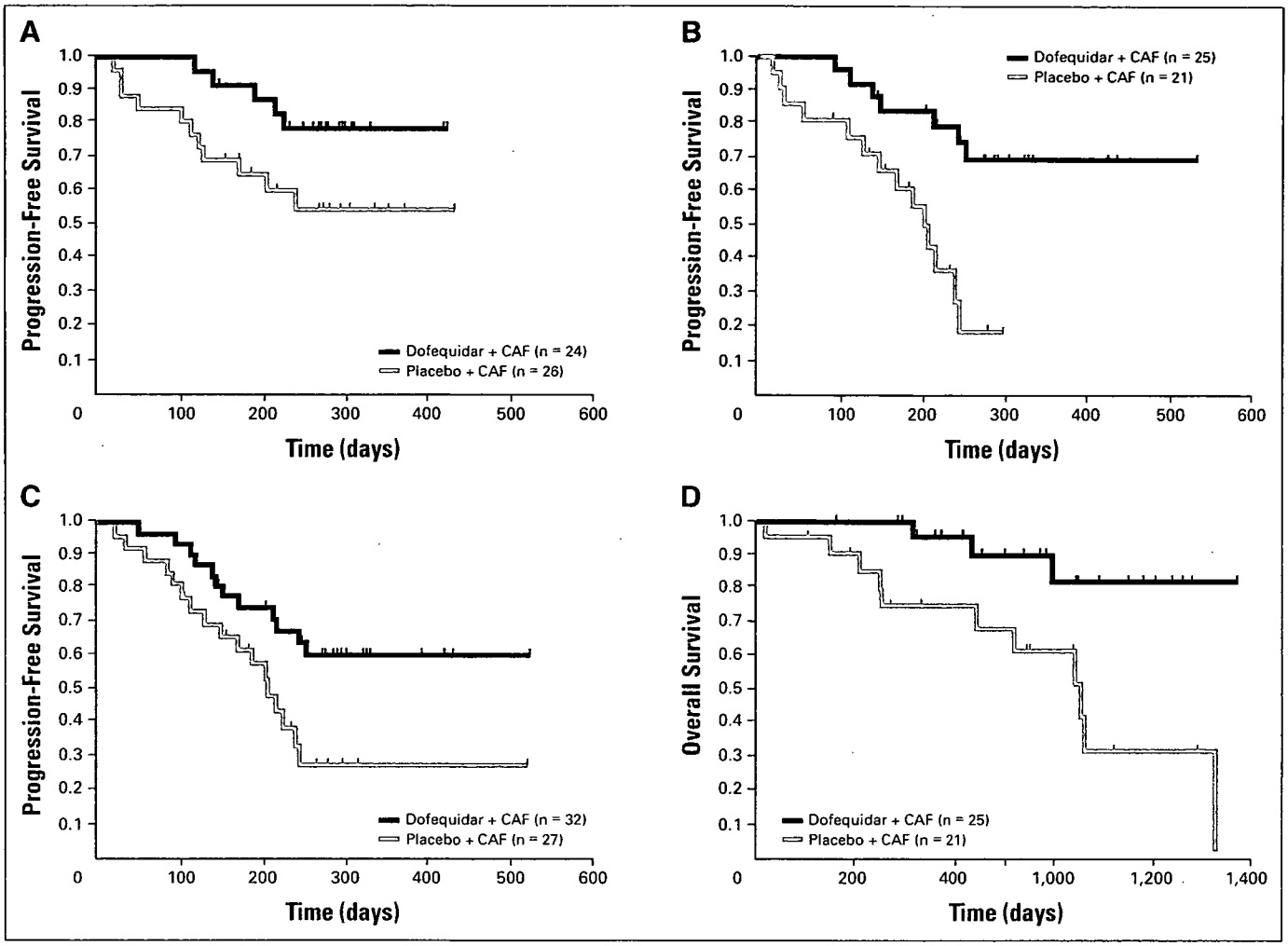

Fig 3. Subgroup analyses. (A) Progression-free survival in premenopausal patients (P = .046); (B) progression-free survival in patients who had no prior therapy (P = .0007); (C) progression-free survival in patients who were stage IV at diagnosis with an intact primary tumor (P = .017); and (D) overall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

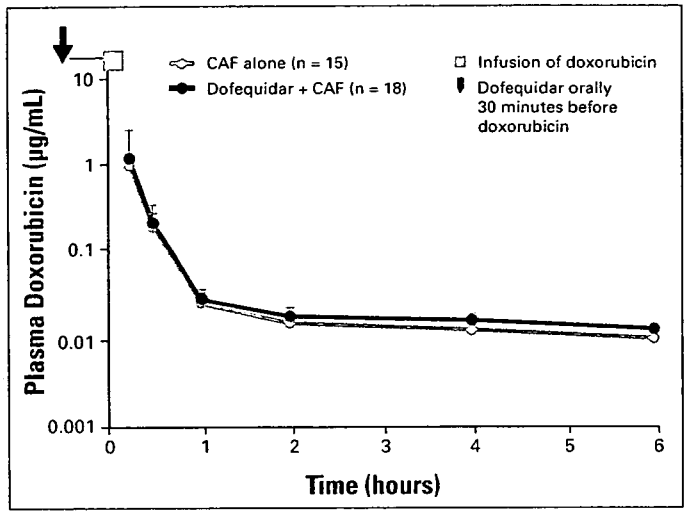

Fig 4. Plasma levels of doxorubicin in patients receiving dofequidar or placebo. CAF, cyclophosphamide, doxorubicin, and fluorouracil.

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®).

Genetic polymorphisms of *CYP2B6* affect the pharmacokinetics/pharmacodynamics of cyclophosphamide in Japanese cancer patients

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Objective To evaluate the effects of genetic polymorphisms of drug metabolizing enzymes on the pharmacokinetics of cyclophosphamide and its active metabolite, 4-hydroxycyclophosphamide, and on the pharmacodynamics.

Experimental Design One hundred and three Japanese patients with malignant lymphoma or breast cancer treated with cyclophosphamide (500–750 mg/m^2) participated in this study. The plasma concentrations of cyclophosphamide and 4-hydroxycyclophosphamide were determined by high-performance liquid chromatography, and pharmacokinetic parameters were calculated. The genotypes of *CYP2B6*, *CYP2C19*, *CYP3A4*, *CYP3A5*, *ALDH1A1*, *GST* genes were determined by allele-specific polymerase chain reaction or polymerase chain reaction–restriction-fragment length polymorphism.

Results A large interindividual difference (54-fold) was observed in the area under the curve ratio of 4-hydroxycyclophosphamide/cyclophosphamide calculated as the metabolic index. We first proved that leukocytopenia and neutropenia were significantly ($P<0.01$) related to the area under the curve of 4-hydroxycyclophosphamide. We found that the homozygotes of *CYP2B6*6* (Q172H and K262R) showed significantly ($P<0.05$) higher clearance and shorter half-life of cyclophosphamide than heterozygotes and homozygotes of *CYP2B6*1*. The small sample size, however, limited the impact. On the other hand, it was clearly demonstrated that the patients possessing the single nucleotide polymorphisms of the *CYP2B6* gene, g.−2320T>C, g.−750T>C (5′-flanking region), g.15582C>T (intron 3), or g.18492T>C (intron 5), had significantly lower area under the curve ratios of 4-hydroxycyclophosphamide/cyclophosphamide, indicating a decreased cyclophosphamide 4-hydroxylation. Of particular importance was the finding that leukocytopenia was significantly related to the single nucleotide polymorphisms g.−2320T>C, g.−750T>C, and g.18492T>C in *CYP2B6* gene, which are highly linked. No relationship was observed between the pharmacokinetics of cyclophosphamide or 4-hydroxycyclophosphamide and genetic polymorphisms of the other enzymes.

Conclusions We clarified that the single nucleotide polymorphisms in the promoter region or introns in the *CYP2B6* affect the potency of cyclophosphamide activation to 4-hydroxycyclophosphamide. This information would be valuable for predicting adverse reactions and the clinical efficacy of cyclophosphamide. *Pharmacogenetics and Genomics* 17:431–445 © 2007 Lippincott Williams & Wilkins.

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Keywords: anticancer drug, chemotherapy, cytochrome P450, genetic polymorphisms, interindividual differences

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Introduction

Cyclophosphamide (CPA) is one of the most widely used anticancer drugs. It is a prodrug that requires metabolic activation to manifest its cytotoxic activity. After the administration of CPA, 75–80% of the dose is metabolized to 4-hydroxycyclophosphamide (4-OH-CPA) (Fig. 1) by CYP2B6, CYP2C19, CYP3A4, and CYP3A5 [1–4]. Among them, CYP2B6 has the highest intrinsic clearance for CPA 4-hydroxylation [3,4]. CYP3A4 is exclusively involved in the *N*-dechloroethylation of CPA [4]. The primary metabolite, 4-OH-CPA, is in equilibrium with its open-ring tautomer aldophosphamide, which undergoes chemical decomposition to form phosphoramide mustard, a bifunctional DNA alkylator and the ultimate cytotoxic metabolite, and acrolein [5,6]. Alternatively, the 4-OH-CPA and aldophosphamide are detoxified by glutathione S-transferase (GST) multigene family with thiols or sulfates [7] and by aldehyde dehydrogenase (ALDH) to carboxycyclophosphamide [8], respectively. Thus, the hepatic metabolism is the dominant route of CPA elimination.

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