drinking increased the risk of lung cancer compared with nondrinkers who never smoked, particularly the OR for heavy smokers (>37 pack-years) and drinkers, which was 8.4 (95% CI, 2.3–30.2; $P = .0012$) in the light drinkers and 7.0 (95% CI, 2.1–23.4) in the heavy drinkers (data not shown).

The involvement of alcohol in lung cancer etiology has been controversial, although many epidemiologic studies have suggested positive associations between different parameters of alcohol consumption and lung cancer risk. In the current study, we have demonstrated that drinking is a strong risk factor for lung cancer that is dose-dependent and is stronger in men than in women. This same tendency was observed even in the genotype analysis, but none of the results indicated a significant association between lung cancer and drinking in women. Furthermore, no associations were observed between peripheral lung adenocarcinoma, drinking, and genotypes of alcohol metabolite-related enzymes in women.

The question of ethnicity in the distribution of the polymorphisms of these alcohol metabolite-related enzyme genes always must be considered. The ADH_3^2 allele is present in almost 60% of whites but is far more rare (5–10%) in Japanese. In contrast, the $ALDH_2^2$ allele is found only in Asians. The *CYP2E1* c2 allele is present in 35% to 56% of Japanese and Chinese, and in 2% to 5% of whites. In the current study, the frequency of variant alleles of each polymorphism was 9.9% for ADH_3, 40.5% for $ALDH_2$, and 41.3% for *CYP2E1*. This is consistent with previous studies in Japanese and other Asians.

We observed that the risk for lung cancer was increased significantly by alcohol consumption in a dose-dependent fashion in individuals with the $ALDH_2^2$ alleles. Previously, some Japanese studies also showed a strong genetic and environmental interaction between $ALDH_2^2$ and alcohol intake for the risk of developing esophageal and upper aerodigestive tract cancer.[18–21] In contrast, for individuals with the $ALDH_2^{1-1}$ genotype, there was an inverse association between alcohol consumption and the risk of lung cancer. These results suggest that increased acetaldehyde concentrations from a reduction in acetaldehyde oxidation caused by the presence of the $ALDH_2^2$ allele contribute to the development of lung cancer. Significantly higher blood acetaldehyde concentrations after drinking in individuals with the ADH_3^1 or $ALDH_2^2$ allele have been reported compared with the concentrations in individuals who lacked these alleles,[11,29] and it has been demonstrated that breath acetaldehyde levels are proportional to blood acetaldehyde levels. Indeed, Muto et al.[30] and Jones[31] observed significantly higher acetaldehyde levels in the breath from individuals with the $ALDH_2^2$ allele than in those without that allele. Therefore, exposure to higher concentrations of acetaldehyde in the lower respiratory tract may play a critical role in alcohol-related carcinogenesis. Regarding the influence of smoking, when adjusted for age, sex, and amount of alcohol consumed, the risks for developing lung cancer in current smokers were 1.5-fold greater for those with the inactive $ALDH_2$ genotype (data not shown) compared with nonsmokers. The lung cancer risk for individuals with the $ALDH_2^2$ allele was not increased further by smoking.

Although there have been some reports of a significant association between the ADH_3^1 allele and some types of upper aerodigestive tract cancer, this association has been controversial.[16,17,32–34] We failed to observe an association between ADH_3 gene polymorphisms and the development of lung cancer, most likely because of the limited statistical power from the low frequency of the variant allele in the Japanese population.

Several investigations[24,31,35,36] have indicated that the *CYP2E1* c2 allele is associated with susceptibility to some types of cancer. However, other investigators reported that carriers of the c2 allele had decreased susceptibility to a number of cancers[25–27,37] and reported no association between *CYP2E1* genotypes and cancer.[23,28,38] Discrepancies among these results may be caused by several factors, including differences in study design, sample size, and the populations' ethnicity. Statistical power usually is very limited in studies of the white population because of the extreme rarity of variant genotypes. Although *CYP2E1* enzyme activity is induced by certain chemicals, such as ethanol, large interindividual variation has been observed in its constitutive activity as well as after induction. Watanabe et al.[39] and Hayashi et al.[15] reported that the *Rsa*I variant c2 allele produced higher enzyme activity than the c1/c1 genotype in Japanese individuals, although this finding is itself controversial.[40–42] Highly activated *CYP2E1* induced by alcohol may play a more important role in the metabolic activation of several tobacco-specific procarcinogens, including various nitrosamines. It has been suggested that these low-molecular-weight carcinogens are associated with the development of peripheral adenocarcinoma. This finding is consistent with the results from our analysis of *CYP2E1* presented in Table 5. However, the *CYP2E1* c2/c2 genotype is not in Hardy-Weinberg equilibrium in the control population, the observed frequencies most likely are underestimates, and these findings of

an association with histologic type most likely are false-positive results. In our analysis of *$ALDH_2$*, the incidence of adenocarcinoma was high among individuals who had the wild-type genotype. Although a high incidence of squamous cell carcinoma was not observed, this result may imply that carcinogenesis caused by acetaldehyde occurs more in cancers other than adenocarcinoma as well as in esophageal and upper aerodigestive tract cancers.

A previous hospital-based study that was conducted in Japan failed to identify any association between the *Rsa*I polymorphism and lung cancer, even when the analysis was stratified according to different histologic type.[28] A more recent study indicated that there was a significant decrease in overall lung cancer risk associated with the possession of at least 1 copy of the *CYP2E1 Rsa*I variant allele, whereas there was no association between the *CYP2E1 Rsa*I polymorphism and the histologic type of lung cancer.[27] However, none of the previous studies had adjusted for risk according to alcohol consumption levels, which strongly influence the activity of this enzyme. In the current study, we demonstrated that there is a difference between individuals who have the *CYP2E1 Rsa*I c2/c2 genotype compared with individuals who have the common c1/c1 genotype, with an adjusted OR of 4.66 (95% CI, 1.36–16.0) for the former group. Because of the low incidence of homozygosity in controls, the genotype distribution was not in Hardy-Weinberg equilibrium in our control population. The increased lung cancer risk among individuals with the *CYP2E1* c2/c2 genotype likely was a false-positive result.

A correlation between the amount of alcohol consumed, genetic polymorphisms in the alcohol metabolite-related enzymes, and the stage of lung cancer was not observed in the current study, and we could not confirm that these factors were related to the aggressiveness of lung cancer. Furthermore, no associations were identified between the location of the primary cancer, the amount of alcohol consumed, and the genotype of these enzymes or between the risk for lung cancer and the type of alcoholic beverage consumed.

In summary, we report a significant association between amounts of alcohol consumed and susceptibility to lung cancer and that the risk of lung cancer in individuals with *$ALDH_2$* variant alleles, but not with *ADH_3* or *CYP2E1* variant alleles, apparently was enhanced more by alcohol intake than in individuals with common genotypes. Moreover, to our knowledge, this is the first report documenting an association between lung cancer and genetic polymorphisms of alcohol metabolite-related enzymes. Because the sample size was relatively small for the investigation of effects stratified by each genotype, the current findings should be confirmed in large-scale studies with greater statistical power.

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Phase II trial of carboplatin and paclitaxel in non-small cell lung cancer patients previously treated with chemotherapy☆

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Summary The purpose of this phase II trial was to evaluate the efficacy and toxicity of carboplatin plus paclitaxel in the treatment of advanced non-small cell lung cancer (NSCLC) previously treated with chemotherapy. Patients with a performance status (PS) of 0 or 1 who had received one or two previous chemotherapy regimens for advanced NSCLC were eligible. Paclitaxel 200 mg/m^2 was infused over 3 h and followed by carboplatin (area under the curve 6) infusion over 1 h, once every 3 weeks. Thirty patients were enrolled. A complete response was observed in 1 patient and a partial response in 10 patients, for an overall response rate of 36.7%. The median time to progression was 5.3 months. The median survival time was 9.9 months, and the 1-year survival rate was 47%. Hematological toxicity in the form of grade 3/4 neutropenia occurred in 54%, but grade 3 febrile neutropenia developed in only 3%. Non-hematological grade 3 toxicities were less frequent. There were no treatment-related deaths. The combination of carboplatin plus paclitaxel is an active and well-tolerated regimen for the treatment of NSCLC patients who have previously been treated with chemotherapy and have a good PS.

1. Introduction

Lung cancer remains a major cause of death from cancer in many countries. More than half of all patients diagnosed with non-small cell lung cancer (NSCLC) have advanced stage IIIB or IV disease at presentation, and patients with advanced NSCLC are candidates for systemic chemotherapy. Platinum-based chemotherapy is considered the standard first-line treatment for patients with advanced NSCLC, and prolongs survival, palliates symptoms, and improves quality of life [1,2]. Many patients with good performance status (PS) when progression occurs after first-line chemotherapy are suitable candidates for second-line chemotherapy [3].

The taxanes are an important class of new agents for the treatment of advanced NSCLC. Paclitaxel, in combination with carboplatin, is the most common regimen

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used as first-line chemotherapy for advanced NSCLC, and this combination has a more favorable toxicity profile and is more convenient to administer than other platinum-based regimens [4,5]. Docetaxel has been investigated more extensively than any other agent for second-line treatment of advanced NSCLC, and the results of two randomized phase III trials of second-line chemotherapy in patients with advanced NSCLC demonstrated that docetaxel monotherapy significantly improved survival compared with best supportive care or other single agents (vinorelbine or ifosfamide) [6,7].

Belani et al. recently reported that results of a phase III trial comparing a carboplatin plus paclitaxel regimen with a cisplatin plus etoposide regimen for first-line treatment of advanced NSCLC [8]. Carboplatin plus paclitaxel yielded a higher response rate (23% versus 15%), time to progression (121 days versus 111 days), and overall quality of life benefit than cisplatin plus etoposide, but the median survival time was better in the cisplatin plus etoposide arm than in the carboplatin plus paclitaxel arm (274 days and 233 days, respectively [$P=0.086$]). The authors reported that a substantially greater proportion of patients in the cisplatin plus etoposide arm received second-line chemotherapy with a taxane-containing regimen than in the carboplatin plus paclitaxel arm, and suggested that treatment with taxanes in a second-line setting may have had an impact on the survival in their study. Remarkably, more than half of the regimens that were used in the second-line setting of their study consisted of paclitaxel alone or carboplatin plus paclitaxel, not docetaxel. While the efficacy of paclitaxel-containing regimens as first-line chemotherapy for advanced NSCLC has been established in many randomized phase III trials [9], the data on the efficacy of paclitaxel-containing regimens in second-line settings are limited [10,11].

Based these considerations we conducted a phase II trial to evaluate the efficacy and toxicity of carboplatin plus paclitaxel in the treatment of advanced NSCLC previously treated with chemotherapy.

2. Patients and methods

2.1. Eligibility criteria

The inclusion criteria were: pathologically confirmed advanced NSCLC patients with measurable disease who had received one or two previous chemotherapy regimens for their disease. Patients were required to submit evidence of failure of prior chemotherapy. Patients who were previously treated with carboplatin or paclitaxel were excluded if the best response was progressive disease (PD). Patients who had received prior radiotherapy were eligible provided that at least 30 days had elapsed between the completion of radiotherapy and entry into the study. Patients were also required to be 20–75 years of age, have an Eastern Cooperative Oncology Group PS of 0 or 1, and have adequate organ function as indicated by the following parameters: absolute neutrophil count $\geq 1500\,mm^{-3}$, platelet count $\geq 100{,}000\,mm^{-3}$, hemoglobin $\geq 9.0\,g/dl$, AST and ALT $\leq 2.0 \times$ the institutional upper normal limits, total bilirubin $\leq 1.5\,mg/dl$, creatinine $\leq 1.5\,mg/dl$, $PaO_2 \geq 65$ Torr. Exclusion criteria were: uncontrolled pleural or pericardial effusion, active concomitant malignancy, prior irradiation to areas encompassing more than a third of the pelvis plus spine, active infection, myocardial insufficiency or myocardial infarction within the preceding 6 months, uncontrolled diabetes mellitus or hypertension, any other condition that could compromise protocol compliance, pregnancy and/or breast-feeding. All patients were required to provide written informed consent before entry into the study. The study was approved by the institutional review board of our institution.

2.2. Treatment plan

Treatment was started within a week of entry into the study. Patients received paclitaxel $200\,mg/m^2$ diluted in 500 ml of 0.9% saline as a 3-h intravenous infusion followed by carboplatin (area under the curve [AUC] 6; Calvert formula) diluted in 250 ml of 5% glucose as a 1-h intravenous infusion, every 3 weeks. All patients were premedicated with dexamethasone (24 mg i.v.), famotidine (20 mg i.v.), and diphenhydramine (50 mg orally) 30 min before the paclitaxel infusion to prevent a hypersensitivity reaction. A 5-HT3-receptor antagonist was intravenously administered as an antiemetic before carboplatin. Therapy was continued for at least two cycles unless the patient experienced unacceptable toxicity or had PD. The maximum number of cycles of chemotherapy was six. In the event of grade 4 leukopenia or thrombocytopenia or of grade 3 neutropenic fever, the dose of carboplatin and paclitaxel was reduced to AUC 5 and $175\,mg/m^2$, respectively, in the following cycle of chemotherapy. The next cycle of chemotherapy was started if the neutrophil count was $\geq 1500\,mm^{-3}$, the platelet count $\geq 100{,}000\,mm^{-3}$, AST and ALT $\leq 100\,IU/l$, total bilirubin $\leq 2.0\,mg/dl$, creatinine $\leq 1.5\,mg/dl$, PS 0 or 1, and the patient was afebrile.

Pretreatment evaluation included a medical history, a physical examination, vital signs, height and body weight, PS, complete blood count, biochemical studies, arterial blood gas analysis, electrocardiogram, chest radiograph and computed tomography scan (CT), abdominal ultrasound or CT, and brain magnetic resonance imaging or CT. A complete blood count, biochemical studies, and chest radiograph were performed weekly during the first cycle of chemotherapy, and 2 weekly starting with the second cycle.

2.3. Response and toxicity assessment

Objective tumor response was assessed as complete response (CR), partial response (PR), stable disease ≥ 8 weeks (SD), or PD according to the Response Evaluation Criteria in Solid Tumors. Measurable lesions were defined as lesions whose longest diameter was $\geq 2\,cm$. Imaging studies were repeated every 4 weeks until the objective tumor response was confirmed. All responses were reviewed by an independent radiologist. Toxicity was graded using National Cancer Institute-Common Toxicity Criteria version 2.0.

2.4. Statistical analysis

The primary endpoint of this study was the response rate, defined as the proportion of patients whose best response was CR or PR among all enrolled patients in the intent-to-treat analysis. The secondary end points were toxicity and overall and progression-free survival (PFS) from the date of enrollment in this study.

According to Simon's minimax two-stage phase II study design, the treatment program was designed for a minimal response rate of 5% and to provide a significance level of 0.05 with a statistical power of 80% in assessing the activity of the regimen according to a 20% response rate. The upper limit for first-stage drug rejection was no response in 13 evaluable patients. The upper limit for second-stage drug rejection was three responses in 27 evaluable patients. Overall survival time was defined as the interval between enrollment in this study and death or the most recent follow-up visit. PFS was defined as the interval between enrollment in this study and the first documented PD, death, or the most recent follow-up visit. Survival was estimated by the Kaplan–Meier analysis method. All comparisons between proportions were performed by Fisher's exact test.

3. Results

3.1. Patient characteristics

Between October 2002 and November 2003, 30 patients were enrolled in this study, and their characteristics are shown in Table 1. Twenty-six (87%) patients were men, and 21 (70%) patients had adenocarcinoma. Median age was 60 years. The majority of the patients (93%) had received prior platinum-based chemotherapy, and seven (23%) patients had received two prior chemotherapy regimens. The platinum-based chemotherapy regimens that had been used were: cisplatin plus vinorelbine ($n = 26$), cisplatin plus gemcitabine ($n = 1$), and carboplatin plus gemcitabine ($n = 1$). There were 15 (50%) responders to any of the prior chemotherapy regimens and 12 of them had experienced a response (CR/PR) to cisplatin-based chemotherapy. Twenty-one (70%) patients had a treatment-free interval of 3 or more months since the final dose of the prior chemotherapy regimen.

A total of 94 cycles of chemotherapy were administered, and the median number of cycles per patient was three (range, 1–6). Four patients had received only one cycle of treatment either because of toxicity (two patients, grade 3 rash), the patient's refusal (one patient), or PD (one patient).

3.2. Response and survival

Two patients were not evaluable for response because the protocol treatment had been terminated because of toxicity (grade 3 rash) during the first cycle of chemotherapy, and they subsequently received further chemotherapy without PD. There was 1 CR and 10 PRs among the 30 patients, and the objective response rate in the intent-to-treat analysis was 36.7% (95% confidence interval [CI], 19.9–56.1%) (Table 2). Treatment outcomes of all patients are listed in

Table 1 Patient characteristics

Characteristic	No. of patients (%)
Patients enrolled	30
Sex	
Male	26
Female	4
Age, years	
Median	60
Range	39–75
ECOG performance status	
0	7
1	23
Stage	
IIIB	11
IV	19
Histology	
Adenocarcinoma	21
Squamous cell carcinoma	7
Large cell carcnioma	2
Prior treatment	
Platinum-based chemotherapy	28 (93)
Docetaxel	5 (16)
Chest radiotherapy	4 (13)
No. of prior chemotherapy regimens	
1	23
2	7

Table 3. The response rate of patients who experienced a response (CR/PR) to prior cisplatin-based chemotherapy was 43% (6/14), as opposed to 23% (3/13) among the non-response patients ($P = 0.41$). The response rate of the patients who had received one prior chemotherapy regimen was 39% (9/23), as opposed to 28% (2/7) among the patients who had received two regimens ($P > 0.99$). According to the treatment-free interval since the final dose of the prior chemotherapy regimen, the response rate of patients whose interval was 3 months or more was 33% (7/21), com-

Table 2 Treatment efficacy ($n = 30$)

	No. of patients	%
Response		
Overall response rate	11	36.7
Complete response	1	3.3
Partial response	10	33.3
Stable disease	12	40
Progressive disease	5	16.7
Not evaluable	2	6.7
Survival		
Median (months)	9.9	
1 year (%)	47	
Progression-free survival		
Median (months)	5.3	

Table 3 Treatment outcomes of all patients

Patient No.	Prior first-line therapy		Prior second-line therapy		Time from last therapy (months)	CBDCA + PTX, best response	PFS (months)	Survival (months)
	Regimen	Best response	Regimen	Best response				
1	CDDP + VNR	SD	DOC	PD	1.8	SD	1.4	25.2
2	CBDCA + GEM	NE	Gefitinib	PD	0.8	PR	3.8	8.8
3	CDDP + VNR	SD			6.8	SD	7.6	18.1
4	CDDP + GEM	PR			9.5	PR	7.5	33.8+
5	CDDP + VNR	SD			4.8	SD	2.8	7.0
6	CDDP + VNR + DOC + RT	PR			6.0	PR	8.0	21.6
7	GEM + VNR	SD			23.0	PD	1.2	7.8
8	CDDP + VNR + RT	PR			13.6	SD	6.7	25.0+
9	CDDP + VNR	SD			5.0	SD	2.1	3.7
10	CDDP + VNR	SD			5.0	PD	1.2	6.7
11	CDDP + VNR	PR			8.9	NE	1.1	3.3
12	CDDP + VNR	SD	Gefitinib	CR	1.9	SD	6.3	6.3
13	CDDP + VNR	PR			5.4	NE	1.0	13.4
14	CDDP + VNR	PR			1.7	SD	4.8	5.7
15	CDDP + VNR + RT	PR			9.3	SD	5.0	15.7
16	CDDP + VNR	SD			2.8	PR	3.7	15.8
17	CDDP + VNR	SD	DOC + GEM	SD	3.8	SD	5.3	21.6+
18	CDDP + VNR + DOC + RT	PR			3.9	SD	4.5	9.0
19	CDDP + VNR	PR			12.9	PR	9.4	16.0
20	CDDP + VNR	PR			11.5	CR	24.8+	24.8
21	CDDP + VNR	PD			1.1	PR	9.2	23.6+
22	CDDP + VNR	SD	DOC	SD	4.5	PD	2.3	5.5
23	Gefitinib	SD			0.9	PR	8.8	12.7
24	CDDP + VNR	PR			11.1	PR	5.3	10.2
25	CDDP + VNR	PR	Gefitinib	PR	4.4	PR	5.5	9.9
26	CDDP + VNR	NE			11.7	PR	7.0	12.2
27	CDDP + VNR	PR			5.4	SD	6.2	9.4
28	CDDP + VNR	SD			0.8	PD	1.4	2.5
29	CDDP + VNR	PR			4.4	PD	0.2	8.4
30	Gefitinib	PD	CDDP + VNR	PD	0.9	SD	3.1	3.3

CBDCA, carboplatin; PTX, paclitaxel; PFS, progression-free survival; CDDP, cisplatin; VNR, vinorelbine; GEM, gemcitabine; DOC, docetaxel; RT, chest radiotherapy; SD, stable disease; NE, not evaluable; PR, partial response; PD, progressive disease; CR, complete response.

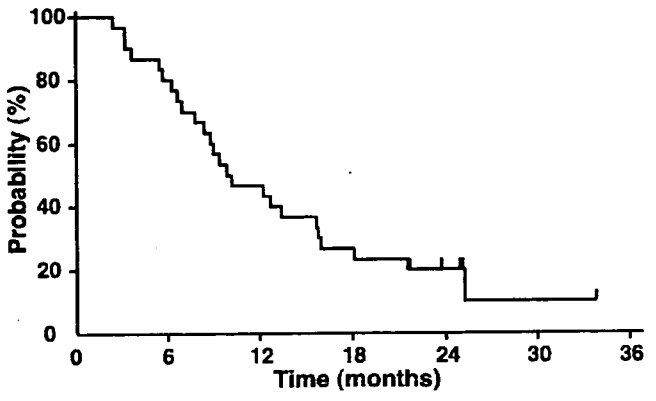

Fig. 1 Kaplan–Meier curve for overall survival.

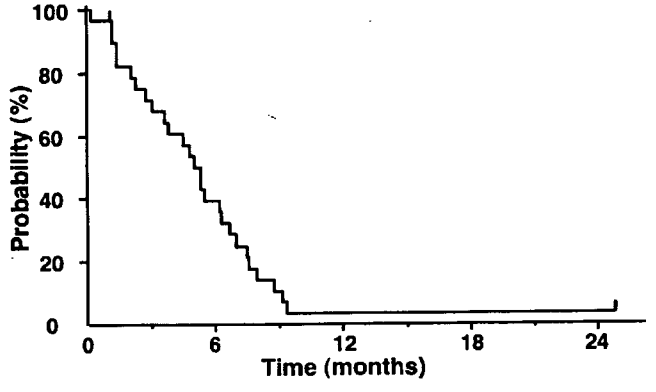

Fig. 2 Kaplan–Meier curve for progression-free survival.

pared with 44% (4/9) in patients in whom it was less than 3 months ($P = 0.68$).

The median follow-up time was 24 months. The median survival time (MST) was 9.9 months (range, 2.5–33.8 months), and the 1-year survival rate was 47% (95% CI, 29–65%). The median PFS was 5.3 months. The Kaplan–Meier curve for overall survival and for PFS is shown in Figs. 1 and 2, respectively. Nineteen patients (63%) received at least one subsequent chemotherapy regimen, and their regimens are shown in Table 4. Fourteen of them were treated with gefitinib, and a PR was achieved in three of them.

3.3. Toxicity

The common toxicities associated with carboplatin plus paclitaxel are listed in Table 5. Grade 3/4 neutropenia occurred in 54% of the patients in our study, but grade 3 febrile neutropenia developed in only 3%. Grade 3/4 anemia and thrombocytopenia were observed in five patients (16%) and two patients (13%), respectively. Non-hematological grade 3 toxicities were less frequent. Grade 3 hyponatremia was observed in five (16%) patients, but they were all asymptomatic. Grade 2 neuropathy occurred in 33% of the patients. There were no treatment-related deaths.

Table 4 Post-study chemotherapy

Regimen	No. of patients	Responder (%)
Gefitinib	14	3 (21)
Docetaxel	9	0
Gemcitabine plus vinorelbine	1	0

4. Discussion

Docetaxel, pemetrexed, and erlotinib have been approved for second-line treatment of advanced NSCLC on the basis of the results of phase III trials [6,7,12,13]. Hanna et al. reported a phase III study comparing 3-weekly pemetrexed 500 mg/m^2 with 3-weekly docetaxel 75 mg/m^2 as second-line treatment for advanced NSCLC. The overall response rate with pemetrexed and docetaxel was 9.1% and 8.8%, respectively, and MST was 8.3 months and 7.9 months, respectively. Although efficacy in terms of the outcome as measured by survival time and response rate was similar for both treatments, the pemetrexed group experienced less grades 3–4 hematological toxicity and alopecia of all grades [12]. In the trial reported by Shepherd et al. 731 NSCLC patients previously treated with chemotherapy were randomized to receive either erlotinib at a dose of 150 mg daily or placebo, and the response rate in the erlotinib group was 8.9%. MST was 6.7 months in the erlotinib group and 4.7 months in the placebo group ($P < 0.001$). The results of their trial showed that erlotinib significantly prolonged the survival of patients with advanced NSCLC who had previously been treated with chemotherapy [13]. Despite the positive results of these phase III trials, the response rate of advanced NSCLC to second-line chemotherapy remains low, and the life expectancy of advanced NSCLC patients remains short. Alternative effective chemotherapy option is needed for second-line treatment of advanced NSCLC.

The combination of carboplatin plus paclitaxel has proved effective as one of the standard platinum-based doublet regimens for first-line treatment of advanced NSCLC [4,5,14]. However, since the efficacy of carboplatin plus paclitaxel used in a second-line setting had hardly been assessed, in the present study we evaluated the efficacy and toxicity of carboplatin plus paclitaxel in the second- or third-line treatment of advanced NSCLC. The results in the 30 patients with advanced NSCLC previously treated with chemotherapy indicated that the combination of carboplatin plus paclitaxel yielded an objective response rate of 36.7% and an MST of 9.9 months, with a 1-year survival rate of 47%. Our study had not included patients who were treated with the platinum/taxane combination chemotherapy. Most of the toxicity observed in our study was hematological. Grade 3/4 neutropenia, anemia, or thrombocytopenia occurred in 54, 16, or 13% of the patients in our study, respectively. Hematological toxicity of carboplatin plus paclitaxel used in first-line treatment for Japanese patients with advanced NSCLC has been reported that grade 3/4 neutropenia, anemia, or thrombocytopenia occurred in 88, 15, or 11% of the patients [15]. The toxicity observed in our study appeared similar to that of carboplatin plus paclitaxel, which was administered as the first-line treatment, although the number of patients in our study was not large. The combination of carboplatin plus paclitaxel seems to be effective and tolerable, not only as first-line therapy for advanced NSCLC but

Table 5 Hematological and non-hematological toxicity ($n = 30$)

Toxicity	NCI-CTC Version 2.0, grade							
	0–1		2		3		4	
	n	%	*n*	%	*n*	%	*n*	%
Leukopenia	11	37	10	33	9	30	0	0
Neutropenia	10	33	4	13	14	47	2	7
Anemia	7	23	18	60	3	10	2	7
Thrombocytopenia	27	90	1	3	2	7	0	0
Febrile neutropenia	29	97	–		1	3	0	0
Nausea	27	90	3	10	0	0	–	
Fatigue	30	100	0	0	0	0	0	0
Neuropathy	20	67	10	33	0	0	0	0
Arthralgia	21	70	8	27	1	3	0	0
Rash	28	93	0	0	2	6	0	0
Infection	29	97	0	0	1	3	0	0
Arrhythmia	29	97	0	0	1	3	0	0
Alopecia	21	70	9	30	–		–	
AST/ALT	29	97	1	3	0	0	0	0
Hyponatremia	25	83	–		5	17	0	0

as second-line therapy as well if the patients had not been previously treated with the platinum/taxane combination chemotherapy.

Hotta at al. reported a meta-analysis based on abstracted data to compare the effect of carboplatin-based chemotherapy with that of cisplatin-based chemotherapy on overall survival, response rate, and toxicity in the first-line treatment of patients with advanced NSCLC [16]. The results indicated that combination chemotherapy consisting of cisplatin plus a third generation agent produced a significant survival benefit compared with carboplatin plus a third generation agent, although the toxicity profiles of the two modalities were quite different. Recently, Pignon et al. reported a pooled analysis from five randomized clinical trials of cisplatin-based chemotherapy in completely resected NSCLC patients [17]. Their analysis suggested that adjuvant cisplatin-based chemotherapy improved survival in patients with NSCLC. Based on the results of their meta-analysis, cisplatin-based chemotherapy should be recommended as first-line therapy for patients with advanced NSCLC. Moreover, in view of the results of our own study, we speculate that the combination of carboplatin plus paclitaxel may be suitable as second-line treatment for advanced NSCLC patients who had experienced progression after first-line cisplatin-based chemotherapy.

Care must be exercised in interpreting the favorable outcome in our study. One concern is that it was a single-institution phase II study, and therefore patient selection may have influenced the outcome. The responders to any of the prior chemotherapy regimens accounted for 50% of the 30 patients enrolled in this study, and about 80% of the patients had received only one prior chemotherapy regimen. The selection criteria, such as an ECOG PS of 0 or 1, may also have contributed to this favorable outcome. Another concern is that our study had included only five patients who were previously treated with chemotherapy using taxanes. Therefore, the efficacy of carboplatin plus paclitaxel as the secondary therapy after chemotherapy using taxanes is not clear. A further randomized study is warranted to be able to draw definitive conclusions about our results.

Conflict of interest statement

None declared.

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Clinical Trials Across Continents: Drug Development Challenges Regarding International Collaborations

By Nagahiro Saijo, MD, PhD

Overview: **A key consideration for global drug development and registration involves the acceptability of foreign clinical data in the different regions. Transcontinental trials could be possible if the clinical trials were done based on the same regulational standard against populations with acceptable ethnic differences. The problems of global drug development are discussed with special stress on pharmacodynamic and pharmacogenomic differences between white and Asian populations.**

THE CANCER burden in developed countries and resource-poor countries is sure to grow for three reasons. First, populations are rapidly increasing worldwide, especially in the majority of poor countries. Second, the elderly proportion is growing in most countries, and third, the incidence and mortality of cancers associated with smoking, diet, and obesity, have been increasing. Despite efforts at early detection and early surgery and radiotherapy, progress in the treatment of such cancers has been very slow, making the development of new anticancer drugs an extremely important and urgent issue to decrease cancer-related deaths worldwide. Resources are so limited that clinical trials need to be conducted as efficiently as possible, and one effort in that direction has been to conduct clinical trials on more than one continent to obtain adequate sample sizes in a short time. Anticancer drug development is a complex process that involves an interplay between industry, academia, government regulatory agencies, patient advocacy groups, and other stakeholders. The goal of anticancer drug development is to simultaneously launch new drugs on the market worldwide. Despite International Conference on Harmonisation (ICH) guideline G5 and the introduction of the bridging strategy, there are major gaps in the dates anticancer drugs become available on the market in different countries, and they do not seem to have dramatically improved.

PROBLEMS IN GLOBAL TRIALS OF ANTICANCER DRUGS

Factors in the complexity of global studies are differences between countries in medical practice, culture, ethnicity, and regulatory policies. The advantages of global development are shorter time for drug development; earlier introduction of new drugs and earlier availability to patients; cost reduction; and reduction in unnecessary exposure of patients to new drugs. The risks of global development are an increase in early-phase clinical trials of many compounds that may fail and may not proceed; low data quality; uncertainty of the acceptability of foreign data; and late-phase clinical trial failure because of unknown ethnic differences in response to the developing compounds.

Factors for success include strategies for global development and each country's development; global team behavior; cultural awareness and communications; and operational delivery. The leader of each global product team should be the worldwide product leader, and each country's leader should provide necessary strategic input into global teams.

The essential factors for team behaviors depend on trust, face-to-face contact, regular communications, open, honest discussion, and ability to challenge.

Factors for the success of global trials include coincidence of strategy for global and local development, the operating team, behaviors, cultural awareness and communications, and power for operational delivery. Ambiguous situations should be avoided by establishing formal rules and procedures. Operational delivery should be transparent, and mutual problems should be shared by global and local investigators. Regular contact by telephone is extremely important. A clear framework and decision making should be made for empowerment for delivery.

Although ICH good clinical practice (GCP) regulations have been distributed to major countries, there are still minor differences between ICH-GCP and local GCP. The requirements are different from local regulatory agencies on preclinical data before initiate clinical trials. Investigators' and patients' understanding of the importance of clinical trials differs by country. The infrastructure for clinical trials, such as the numbers of well-trained investigators and clinical research coordinators are sometimes inadequate, and sometimes there is poor information technology support and training in institutions. The process of applying for permission to conduct a clinical trial and institutional board review differ by institution and are sometimes complicated. English skills sometimes are very poor, and some investigators and institutions cannot accept English documents.

ETHNIC DIFFERENCES

It will be extremely difficult to conduct trials across continents if there are ethnic differences in pharmacokinetics, pharmacodynamics, pharmacogenetics, and pharmacogenomics. Ethnic differences have been clearly demonstrated in regard to only a few anticancer drugs, and progress in pharmacogenomic studies has led to the

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Author's disclosure of potential conflicts of interest is found at the end of this article.

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identification of some of the mechanisms responsible for the ethnic differences.

EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITOR

A phase I Japanese trial of gefitinib revealed five dramatic responders, and the response rate among the 36 patients accrued to the phase I trial was more than 25%. Subsequent global phase II trials, such as Iressa Dose Evaluation in Advanced Lung Cancer (IDEAL) I and IDEAL II, have yielded a higher response rate in a Japanese population (28%) than in a white population (10%).[1,2] In April 2004, extremely important data were reported suggesting that epidermal growth factor receptor (EGFR) mutations, especially deletion of exon 19 and the point mutation of exon 21, determine sensitivity to EGFR tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib.[3,4] The frequency of EGFR mutations has been found to be significantly higher in Asian populations, including Japanese, than in whites (32% vs. 6%). This difference may explain the difference in response rate to EGFR TKIs. The frequency of EGFR mutations also correlated well with clinical factors, such as female sex, nonsmoker, and adenocarcinoma, which are closely related to the response to EGFR TKI.[5,6] The results of the global Iressa Survival Evaluation in Lung Cancer (ISEL) and National Cancer Institute of Canada Clinical Trials Group BR-21 studies also suggest ethnic differences in sensitivity to EGFR TKI.

The ISEL study is a large randomized controlled trial of gefitinib in patients at 210 centers across 28 countries, and the difference between survival time was not statistically significant difference (hazard ratio [HR] = 0.89; $p = 0.087$) between the gefitinib group and placebo group. However, there was a very clear difference in survival between two groups in the Asian population (HR = 0.66; $p = 0.012$), although it consisted of only 342 patients, whereas the survival curves of the gefitinib group and placebo group in the non-Asian population (HR = 0.99; $p = 0.364$) of 1,350 patients were superimposable. In the BR-21 study of erlotinib, the HR for overall survival in the Asian group (0.61) was significantly smaller than in the white group (0.79).[7] These results strongly suggest that EGFR TKIs are different drugs between Asian and whites indicating that different clinical trials of EGFR TKIs should be scheduled based on ethnic differences. Astra Zeneca has instituted the Iressa Pan Asian Study into Asian populations alone. Many global clinical trials have been initiated in Asian countries, including Japan, Korea, China, Taiwan, Singapore, and Thailand. The accrual spread is generally good. If the trials are limited to Asian countries, pharmacogenomic ethnic differences are thought to be small, if they exist at all.

COMMON ARM ANALYSIS

Two common analyses of paclitaxel/carboplatin therapy in advanced non-small cell lung cancer (NSCLC) were presented in American Society of Clinical Oncology Annual Meetings in 2004 and 2006.[8,9] The purpose of these analyses was to evaluate whether the results of cancer clinical trials conducted in Japan can be directly extrapolated to U.S. populations. Potential differences that may influence the results include trial design and conduct, study-specific criteria, patient demographics, and population-based pharmacogenomics. The purpose of common arm analysis is to demonstrate similarities and differences in patient characteristics and outcomes of the same treatment regimen in Japanese and United States trials in advanced-stage NSCLC, to provide a basis for standardization of study design/conduct, to facilitate interpretation of future trials, and to take the first step toward joint National Cancer Institute-sponsored studies in lung cancer between the two countries.

The trials chosen for this analysis were the Four-Arm Cooperative Study (FACS),[10] Japan Multicenter Trial Organization (JMTO), and Southwest Oncology Group (SWOG) lung programs.[11] The conditions for selection were separate phase III trials, but with an identical common treatment regimen in each, prospective design and conduct, common eligibility and staging, and common response and toxicity criteria. SWOG 0003 was a phase III trial of paclitaxel (225 mg/m^2) and carboplatin (area under the time-concentration curve [AUC] = 6) with or without tirapazamine in advanced NSCLC. The FACS trial compared four arms: irinotecan and cisplatin (reference regimen), paclitaxel (200 mg/m^2) and carboplatin (AUC = 6), gemcitabine and cisplatin, and vinorelbine and cisplatin. The JMTO trial was a phase III trial comparing paclitaxel (225 mg/m^2) and carboplatin (AUC = 6) with gemcitabine/vinorelbine followed by docetaxel. In each trial paclitaxel and carboplatin was administered every 3 weeks. Patients were evenly distributed between arms in regard to age, sex, stage, and histology.

Treatment delivery consisted of a median number of cycles of three, four, and four in the FACS trial, S0003 trial, and JMTO trial, respectively, and the percentage of patients who received more than three cycles was significantly lower in the FACS trial than in the S0003 trial. The JMTO LC00–03 trial whose frequency dose was reduced was significantly higher than in the S0003 trial, although the percentage of patients who received more than three cycles was the same. The frequencies of grade 4 neutropenia and febrile neutropenia in the toxicity analysis were significantly higher in the FACS trial and LC00–03 trial than in the S0003 trial, but grade 3 to 4 neuropathy was more frequent in the S0003 trial and LC00–03 trial than in the FACS trial. The response rates in the three trials ranged from 32% to 36% and were almost the same. Progression-free survival time, median survival time, and 1-year survival rates were significantly better in the Japanese trials than in the S0003 trial. This common arm analysis shows great similarities in patient characteristics in the FACS, LC00–03 trial, and S0003 trial. The differences in toxicities may be due to differences in cumulative paclitaxel dose (neuropathy) and/or population-based pharmacogenomics (increased neutropenia and febrile neutropenia in the FACS trial despite lower paclitaxel doses). Survival with paclitaxel/carboplatin was

significantly better in the Japanese trials, although the response rates were equivalent.

The findings discussed here suggest that possible pharmacogenomic differences in drug disposition should be carefully considered in clinical trials across continents.

Sample collection for a pharmacogenomic analysis of taxanes has been completed in Japan. Single nucleotide polymorphism data for key enzyme/protein in the metabolism of taxanes have been obtained, and pharmacokinetics and pharmacodynamics data have also been collected. Differential analysis of the pharmacogenomics of the response to taxanes in the United States and Japan may make it possible to solve the problems of pharmacogenomic differences in clinical trials across continents.

Author's Disclosures of Potential Conflicts of Interest

Author	Employment or Leadership Positions (Commercial Firms)	Consultant or Advisory Role	Stock Ownership	Honoraria	Research Funding	Expert Testimony	Other Remuneration
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Review Article

Genes Regulating the Sensitivity of Solid Tumor Cell Lines to Cytotoxic Agents: A Literature Review

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In order to review gene alterations associated with drug responses *in vitro* to identify candidate genes for predictive chemosensitivity testing, we selected from literature genes fulfilling at least one of the following criteria for the definition of '*in vitro* chemosensitivity associated gene': (i) alterations of the gene can be identified in human solid tumor cell lines exhibiting drug-induced resistance; (ii) transfection of the gene induces drug resistance; (iii) down-regulation of the gene increases the drug sensitivity. We then performed Medline searches for papers on the association between gene alterations of the selected genes and chemosensitivity of cancer cell lines, using the name of the gene as a keyword. A total of 80 genes were identified, which were categorized according to the protein encoded by them as follows: transporters ($n = 15$), drug targets ($n = 8$), target-associated proteins ($n = 7$), intracellular detoxifiers ($n = 7$), DNA repair proteins ($n = 10$), DNA damage recognition proteins ($n = 2$), cell cycle regulators ($n = 6$), mitogenic and survival signal regulators ($n = 7$), transcription factors ($n = 4$), cell adhesion-mediated drug resistance protein ($n = 1$), and apoptosis regulators ($n = 13$). The association between the gene alterations and chemosensitivity of cancer cell lines was evaluated in 50 studies for 35 genes. The genes for which the association above was shown in two or more studies were those encoding the major vault protein, thymidylate synthetase, glutathione S-transferase pi, metallothionein, tumor suppressor p53, and bcl-2. We conclude that a total of 80 *in vitro* chemosensitivity associated genes identified in the literature are potential candidates for clinical predictive chemosensitivity testing.

Key words: chemotherapy – sensitivity – drug resistance – solid tumor

INTRODUCTION

Malignant neoplastic diseases remain one of the leading causes of death around the world despite extensive basic research and clinical trials. Advanced solid tumors, which account for most malignant tumors, still remain essentially incurable. For example, 80% of patients with non-small cell lung cancer have distant metastases either at the time of the initial diagnosis itself or at the time of recurrence after surgery for the primary tumor. Systemic chemotherapy against malignant tumors remains of limited efficacy in spite of the development in the recent past of several new chemotherapeutic agents; therefore, patients with distant metastases rarely live for long (1).

Tumor response to chemotherapy varies from patient to patient, and clinical objective response rates to standard chemotherapeutic regimens have been reported to be in the range of 20–40% for most common solid tumors. Thus, it would be of great benefit it became possible to predict chemosensitivity of various tumors even prior to therapy. DNA, RNA and protein-based chemosensitivity tests have

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been performed in an attempt to predict the clinical drug response, but the precise gene alterations that might be predictive of the chemosensitivity of the tumors are still unknown. Here we aimed to review the gene alterations that may be associated with the drug response *in vitro* (*in vitro* chemosensitivity associated genes) in order to identify candidate genes for predictive chemosensitivity testing in the clinical setting. The association between these gene alterations and clinical chemosensitivity in lung cancer patients has been reported elsewhere (2).

METHODS

In vitro chemosensitivity associated genes were identified from the medical literature as described previously (2). Briefly, we conducted a Medline search for papers on tumor drug resistance published between 2001 and 2003. This search yielded 112 papers, including several review articles. Manual search of these papers led to identification of 134 genes or gene families that were potentially involved in drug resistance based on their function. We conducted a second Medline search for *in vitro* studies of the 134 genes or gene families using the name of the gene as a keyword. Genes that fulfilled at least one of the following criteria for the definition of *in vitro* chemosensitivity associated gene were selected from the 134 genes: (i) alterations of the gene can be identified in a human solid tumor cell lines exhibiting drug-induced resistance; (ii) transfection of the gene induces drug resistance; (iii) down-regulation of the gene or of the protein encoded by it increases the drug sensitivity. For this last category, we included studies in which the gene expression or function was suppressed by antisense RNA, hammerhead ribozyme, or antibody against the gene product. Finally, a Medline search for papers on the association between gene alterations and chemosensitivity of solid tumor cell lines was performed using the name of the gene as a keyword. Papers in which the association was evaluated in 20 or more cell lines were included in this study. The name of each gene was standardized according to the Human Gene Nomenclature Database of National Center for Biotechnology Information (NCBI).

RESULTS

Of the 134 genes or gene families, gene alterations were found in cells exhibiting drug-induced resistance, transfection of the gene increased or decreased the drug resistance,

Table 1. Transporters and *in vitro* evidence of association with chemosensitivity

Gene symbol	Alterations in DIRC	Sensitivity of		Drugs	Association with chemosensitivity (cancer, drug)	Reference no.
		UCs	DCs			
ABCA2	U	–	S	Estramustine	–	1
ABCB1	U	R	S	DOX, PTX, VCR, VBL	Yes (lung, DOX)	2–11
					No (lung, DOX)	12
ABCB11	–	R	–	PTX	–	13
ABCC1	U	R	S	CPT, DOX, ETP, MTX, VCR	Yes (lung, CDDP, DOX)	11,14–21
					No (lung, PTX)	22
ABCC2	U	R	S	CDDP, DOX, MTX, VCR	No (lung, DOX)	18, 21, 23–25
ABCC3	NC, U	R	–	ETP, MTX	Yes (lung, DOX)	21, 25–28
ABCC4	NC, U	NC, R	–	MTX	No (lung, DOX)	12, 25, 29–31
ABCC5	NC, U	NC	–	DOX, MIT	Yes (lung, ETP)	12, 25, 31–34
ABCG2	M, U	R	–	DOX, MIT, MTX, SN38, TOP	–	35–43
MVP	U	–	NC	DOX	Yes (brain, CDDP, DOX)	44–47
					Yes (lung, DOX)	10
ATP7A	U	–	–	CDDP	–	48
ATP7B	U	R	–	CDDP	–	48–52
SLC29A1	U	–	–	5-FU	No (NCI-panel)	52, 53
SLC28A1	–	S	–	5′-DFUR	No (NCI-panel)	53, 54
SLC19A1	D	S	–	MTX	Yes (NCI-panel)	55–58

Alterations in drug-induced resistance cells (DIRC): D, down-regulated; M, mutated; NC, no change; U, up-regulated. Sensitivity of up-regulating cells (UCs) and down-regulating cells (DCs): NC, no change; R, resistant; S, sensitive.
Drugs: CDDP, cisplatin; CPT, irinotecan; DOX, doxorubicin; ETP, etoposide; MIT, mitoxantrone; MTX, methotrexate; PTX, paclitaxel; SN38, irinotecan metabolite; TOP, topotecan; VBL, vinblastine; 5-FU, 5-fluorouracil; 5′-DFUR, 5′-deoxy-5-fluorouridine, capecitabine metabolite.

Table 2. Drug targets, the associated proteins, and *in vitro* evidence of association with chemosensitivity

Gene symbol	Alterations in DIRC	Sensitivity of		Drugs	Association with chemosensitivity (cancer, drug)	Reference no.
		UCs	DCs			
TUBB	IEC, M	–	–	PTX	–	59–63
TUBB4	U	–	S	PTX	Yes (NCI-panel, PTX)	59, 60, 63–66
TUBA	IEC, M	R	–	PTX	–	64, 67, 68
TYMS	U	R	S	5-FU	Yes (renal cell, 5-FU)	69–74
					No (NCI-panel, 5-FU)	75
					Yes (lung, DOX)	10
TOP1	M	R*	–	CPT	–	76–84
TOP2A	M, D	–	–	ETP, DOX	No (lung, DOX)	10, 82–91
TOP2B	D	–	–	ETP	–	86, 87
DHFR	M, U	R*	–	MTX	–	92–96
MAP4	–	S	–	PTX	–	97
MAP7	–	S	–	PTX	–	98
STMN1	U	R	–	PTX	–	99, 100
KIF5B	–	R	R	ETP, PTX	–	101, 102
HSPA5	–	R	–	ETP	–	103
PSMD14	–	R	–	CDDP, DOX, VBL	–	104
FPGS	D	–	–	5-FU	–	105

Alterations in drug-induced resistance cells (DIRC): D, down-regulated; IEC, isoform expression change; M, mutated; U, up-regulated. Sensitivity of up-regulating cells (UCs) and down-regulating cells (DCs): R, resistant; S, sensitive. Drugs: CDDP, cisplatin; CPT, irinotecan; DOX, doxorubicin; ETP, etoposide; MTX, methotrexate; PTX, paclitaxel; VBL, vinblastine; 5-FU, 5-fluorouracil.
*Over-expression of the mutant gene.

and down-regulation of the gene altered the drug sensitivity for 45, 57 and 32 genes, respectively, and a total of 80 genes fulfilled the criteria for the definition of an '*in vitro* chemosensitivity associated gene'. The genes were categorized according to the protein encoded by them as follows: transporters ($n = 15$, Table 1), drug targets ($n = 8$, Table 2), target-associated proteins ($n = 7$, Table 2), intracellular detoxifiers ($n = 7$, Table 3), DNA repair proteins ($n = 10$,

Table 3. Intracellular detoxifiers and *in vitro* evidence of association with chemosensitivity

Gene symbol	Alterations in DIRC	Sensitivity of		Drugs	Association with chemosensitivity (cancer, drug)	Reference no.
		UCs	DCs			
GSTP1	U	–	S	CDDP, DOX, ETP	Yes (lung, DOX)	10, 106, 107
					Yes (NCI-panel)	108
GPX	–	R, NC	–	DOX	Yes (lung, CDDP)	109–112
GCLC	U	R	S	CDDP, DOX, ETP	Yes (NCI-panel)	106, 108, 113–121
GGT2	U	R	–	CDDP, OXP	–	114, 117, 122, 123
MT	U, NC	R	–	CDDP	Yes (urinary tract, CDDP)	118, 124–130
					Yes (lung, DOX)	10, 131
RRM2	U	R	–	5-FU, GEM, HU	–	71, 132–134
AKR1B1	U	–	–	DNR	–	135

Alterations in drug-induced resistance cells (DIRC): NC, no change; U, up-regulated. Sensitivity of up-regulating cells (UCs) and down-regulating cells (DCs): NC, no change; R, resistant; S, sensitive. Drugs: CDDP, cisplatin; DNR, daunorubicin; DOX, doxorubicin; ETP, etoposide; GEM, gemcitabine; HU, hydroxyurea; OXP, oxaliplatin; 5-FU, 5-fluorouracil.

Table 4. DNA damage recognition and repair proteins and *in vitro* evidence of association with chemosensitivity

Gene symbol	Alterations in DIRC	Sensitivity of		Drugs	Association with chemosensitivity (cancer, drug)	Reference no.
		UCs	DCs			
HMGB1	U	–	–	CDDP	–	136
HMGB2	–	S	–	CDDP	–	137
ERCC1	U	R	S	CDDP	–	138–140
XPA	U	R	–	CDDP	No (NCI-panel)	141–143
XPD	–	R	–	CDDP	Yes (NCI-panel)	142–144
MSH2	D, NC	–	–	CDDP	–	145, 146
MLH1	D, NC	–	–	CDDP	–	145–147
PMS2	D, NC	–	–	CDDP	–	146, 147
APEX1	–	R	–	BLM	–	148
MGMT	–	R	S	CPM, ACNU	Yes (lung, DOX)	10, 149–152
BRCA1	U	S	R	PTX	–	153–155
GLO1	–	R	–	DOX	–	156

Alterations in drug-induced resistance cells (DIRC): D, down-regulated; NC, no change; U, up-regulated. Sensitivity of up-regulating cells (UCs) and down-regulating cells (DCs): R, resistant; S, sensitive. Drugs: ACNU, 1-(4-amino-2-methyl-5-pyrimidinyl)-methyl-3-(2-chloroethyl)-3-nitrosourea; BLM, bleomycin; CDDP, cisplatin; DOX, doxorubicin; PTX, paclitaxel.

Table 4), DNA damage recognition proteins ($n = 2$, Table 4), cell cycle regulators ($n = 6$, Table 5), mitogenic and survival signal regulators ($n = 7$, Table 6), transcription factors ($n = 4$, Table 6), cell adhesion-mediated drug resistance protein ($n = 1$, Table 6), and apoptosis regulators ($n = 13$, Table 7).

The association between the gene alterations and *in vitro* chemosensitivity was evaluated in one study for 25 genes, in two studies for seven genes, in three studies for two genes, and in five studies for one gene, and in a total of 50 studies for 35 genes (Table 8). Significant association was found between chemosensitivity and alterations of genes encoding transporters, drug targets and intracellular detoxifiers (Table 8). Genes for which such association was shown in two or more studies were those encoding the major vault protein/lung resistance-related protein (*MVP*) (Table 1), thymidylate synthetase (*TYMS*) (Table 2), glutathione S-transferase pi (*GSTP1*), metallothionein (*MT*) (Table 3), tumor suppressor protein p53 (*TP53*), and B-cell CLL/lymphoma 2 (*BCL2*) (Table 7).

DISCUSSION

We identified a total of 80 *in vitro* chemosensitivity associated genes. These genes have been the subject of considerable research, and of numerous scientific publications. In addition, we may also have to expect the existence of many other genes associated with chemosensitivity

Table 5. Cell cycle regulators and *in vitro* evidence of association with chemosensitivity

Gene symbol	Alterations in DIRC	Sensitivity of		Drugs	Association with chemosensitivity (cancer, drug)	Reference no.
		UCs	DCs			
RB1	–	R	–	DOX	Yes (lung, DOX)	157–159
					No (lung, CDDP, DOX)	160
GML		S	–	MMC, PTX	Yes (lung, CDDP)	161–163
CDKN1A	U	R, S	S	CDDP, BCNU, PTX	–	164–171
CCNND1	–	R, S	S	CDDP, MTX, PTX	No (lung, DOX)	10, 172–176
CDKN2A	–	S, R	–	CDDP, 5-FU, PTX, TOP	Yes (brain, 5-FU)	177–184
CDKN1B	–	R	–	DOX	–	185

Alterations in drug-induced resistance cells (DIRC): U, up-regulated. Sensitivity of up-regulating cells (UCs) and down-regulating cells (DCs): R, resistant; S, sensitive. Drugs: BCNU, carmustine; CDDP, cisplatin; DOX, doxorubicin; MMC, mitomycin C; MTX, methotrexate; PTX, paclitaxel; TOP, topotecan; 5-FU, 5-fluorouracil.

Table 6. Mitogenic and survival signal regulators, integrins, transcription factors and *in vitro* evidence of association with chemosensitivity

Gene symbol	Alterations in DIRC	Sensitivity of		Drugs	Association with chemosensitivity (cancer, drug)	Reference no.
		UCs	DCs			
ERBB2	–	R, NC	S	CDDP, PTX	Yes (lung, DOX)	10, 22, 186–191
EGFR	–	R	–	DOX	No (lung, CDDP, DOX, PTX)	10, 22, 112, 192
KRAS2	–	R*	–	CDDP	–	193
HRAS	–	R*, NC	–	Ara-C, DOX, PTX	No (lung, DOX)	10, 193–197
RAF1	–	R	–	DOX	–	198
AKT1	–	NC, R	S	CDDP, DOX, PTX	–	199–201
AKT2	–	R	S	CDDP	–	200, 202
ITGB1	–	–	S	ETP, PTX	–	203, 204
JUN	–	R	–	CDDP	No (lung, DOX)	10, 205
FOS	U	R	S	CDDP	No (lung, DOX)	10, 206–208
MYC	NC, U	S, R	R, S, NC	CDDP, DOX	No (lung, DOX)	10, 209–216
NFKB1	U	–	S	5-FU, DOX, ETP	–	217–222

Alterations in drug-induced resistance cells (DIRC): NC, no change; U, up-regulated. Sensitivity of up-regulating cells (UCs) and down-regulating cells (DCs)): NC, no change; R, resistant; S, sensitive. Drugs: Ara-C, 1-beta-D-arabinofuranosylcytosine; CDDP, cisplatin; DOX, doxorubicin; ETP, etoposide; PTX, paclitaxel; 5-FU, 5-fluorouracil.
*Up-regulated with mutated K-ras gene.

Table 7. Apoptosis regulators and in vitro evidence of association with chemosensitivity

Gene symbol	Alterations in DIRC	Sensitivity of		Drugs	Association with chemosensitivity (cancer, drug)	Reference no.
		UCs	DCs			
TP53	–	S, R*	R, S	CDDP, DOX	Yes (brain)	223–229
					Yes (NCI-panel)	230
					No (breast, DOX)	231
					No (breast, DOX, PTX)	232
					No (lung, PTX)	22
MDM2	–	S, R	S	CDDP, DOX, PTX	–	169, 233–238
TP73	–	–	R	CDDP, ETP	–	239, 240
BCL2	U, D	R	–	CDDP, CPT, DOX	Yes (breast, DOX)	164, 198, 231, 241–244
					Yes (lung, PTX)	22
					No (breast, DOX)	232
BCL2L1	NC	R	S	CDDP, PTX	–	243–251
MCL1	–	–	S	DTIC	–	252
BAX	NC	S	R	CDDP, ETP, 5-FU	No (breast, DOX)	231, 244, 253–260
					No (lung, PTX)	22
BIRC4	–	NC	S	PTX	–	261, 262
BIRC5	–	R	S	CDDP, ETP	–	263–265
TNFRSF6	NC	–	S	CDDP	Yes (lung, DOX)	10, 242
CASP3	–	S	–	CDDP, DOX, ETP	No (lung, DOX)	10, 266–268
CASP8	–	–	R	CDDP	–	261
HSPB1	C	R	S	DOX	–	52, 269–273

Alterations in drug-induced resistance cells (DIRC): D, down-regulated; NC, no change; U, up-regulated. Sensitivity of up-regulating cells (UCs) and down-regulating cells (DCs): NC, no change; R, resistant; S, sensitive. Drugs: CDDP, cisplatin; CPT, irinotecan; DOX, doxorubicin; DTIC, dacarbazine; ETP, etoposide; PTX, paclitaxel; 5-FU, 5-fluorouracil.
*Resistant in mutant *TP53* over-expressed cells.

Table 8. Gene categories and association with in vitro chemosensitivity

Category	No. of genes	Total no. of studies	No. of studies showing association (%)
Transporter	15	13	7 (54)
Drug target	8	5	3 (69)
Target associated protein	7	0	0 (0)
Intracellular detoxifier	7	6	6 (100)
DNA repair	10	3	2 (67)
DNA damage recognition protein	2	0	0 (0)
Cell cycle	6	5	3 (60)
Mitogenic signal	5	3	1 (33)
Survival signal	2	0	0 (0)
Transcription factor	4	3	0 (0)
Cell adhesion-mediated drug resistance protein	1	0	0 (0)
Apoptosis	13	12	5 (42)
Total	80	50	22 (44)

but not selected in the current study, because they have never caught the scientific eye for some reasons. Thus, the results of this study may be significantly influenced by publication bias. Nonetheless, we do believe that these genes have been selected reasonably carefully, and that they may be helpful for establishing a clinical predictive chemosensitivity test.

While the association between alterations of the 80 genes and the chemosensitivity of various cell lines was evaluated in 50 studies, significant association was observed in only 22 (44%) (Table 8). The cellular functions of a gene vary among cell types and experimental conditions. The evaluation of the gene functions, however, was conducted under only limited cellular contexts in these studies, as expected. Thus, for example, the conditions of a gene transfection experiment may differ from those of an experiment to evaluate the chemosensitivity for many cell lines. The gene functions may not necessarily be examined under all possible conditions, but the evaluation must be conducted under conditions similar to those in the clinical setting in order to develop clinical chemosensitivity testing using these genes.

The other possibility for the poor correlation to *in vitro* chemosensitivity may be that more than one gene alterations are involved in the chemosensitivity of tumors. This may be discussed from the standpoint of the signal transduction pathway and from the cellular standpoint. From the standpoint of the signal transduction pathway, more than one gene may be involved in the reaction to a cytotoxic agent. One of the best examples is cooperation of *TP53* with another member of the p53 family, p73 (*TP73*), in the response to both DNA damage and chemosensitivity (3,4). From the cellular standpoint, several pathways may work additively, antagonistically, or complementally in determining the chemosensitivity of the cell. This can be understood well from the context of induction and inhibition of apoptosis being controlled by pro-apoptotic and anti-apoptotic pathways. Thus, it would be important to study several pathways at the same time, or to evaluate the net effect of the involvement of various pathways.

Complex factors influencing the cellular chemosensitivity may be operative on a tumor *in vivo*, in such a way that the tumor may exhibit highly heterogeneous gene alterations; that the tumor cells may interact with various host cells, including immune cells, fibroblasts and vascular endothelial cells; and that the differences in the distance between each tumor cell and blood vessels may affect the exposure level of tumor cells to a drug. No systematic approach has been developed to include this complex interplay of factors in the study of cellular chemosensitivity, although studies on cell adhesion-mediated drug resistance may be partly helpful.

Among the six genes for which the association was shown in two or more *in vitro* studies, four encode classical drug resistance proteins which are known to inhibit the drug–target interaction. These proteins are relatively specific for the drug as well as the cell type; e.g. *TYMS* is critical for 5-fluorouracil sensitivity. Thus, *TYMS* is a good candidate for chemosensitivity testing in patients with colorectal cancer who are treated with 5-fluorouracil (Table 2). *MVP* is involved in the transport of doxorubicin, therefore, it would be of interest to examine the association between the expression of *MVP* and the drug response in patients with breast cancer; the association of *MVP* with chemosensitivity has been evaluated only for brain tumor and lung cancer cell lines, to date (Table 1). However, the remaining two of the six genes, *TP53* and *BCL2*, are associated with apoptosis, and therefore may be relatively cell-type specific. Since all the three *in vitro* studies using breast cancer cell lines failed to show any associations between alterations of these genes and the chemosensitivity, the association should be evaluated in other tumor types in the clinical setting (Table 7).

The recently developed cDNA microarray technique allows analysis of the mRNA expression of more than 20 000 genes at once, and as many as 100–400 genes have been statistically shown as potentail chemosensitivity-related genes in various studies (5–7). The 80 genes in the current study were selected theoretically based on their known functions, and their contribution to *in vitro* chemosensitivity was shown in the experiments. Thus, it would be of interest to evaluate the expression profiles of these genes by cDNA microarray analysis, even if the difference in expression between sensitive and resistant cell lines does not reach statistical significance.

In conclusion, 80 *in vitro* chemosensitivity associated genes were identified from a review of the literature, which

may be considered to be future candidates for clinical predictive chemosensitivity testing.

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Conflict of interest statement

None declared.

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