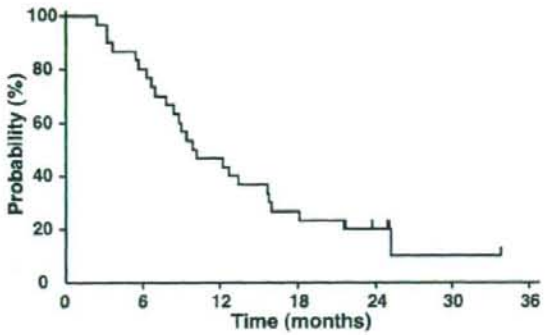

Fig. 1 Kaplan–Meier curve for overall survival.

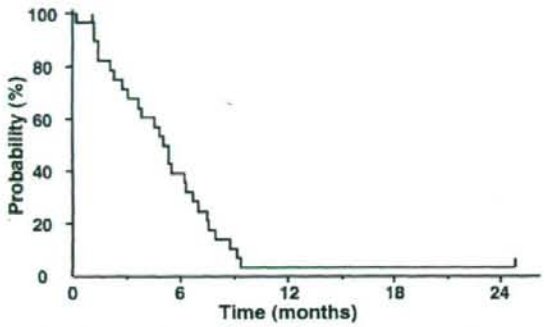

Fig. 2 Kaplan–Meier curve for progression-free survival.

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 viborelbine	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
Nagahiro Saijo			Takeda	Janssen-Cilag; Chugai; Kirin; Takeda; Eisai, Inc; Lilly Oncology; Merck; AstraZeneca			

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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
CCND1	–	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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Irinotecan pharmacokinetics/pharmacodynamics and *UGT1A* genetic polymorphisms in Japanese: roles of *UGT1A1*6* and **28*

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Objectives **SN-38, an active metabolite of irinotecan, is detoxified by glucuronidation with UGT1A isoforms, 1A1, 1A7, 1A9, and 1A10. The pharmacogenetic information on *UGT1A* haplotypes covering all these isoforms is important for the individualized therapy of irinotecan. Associations between *UGT1A* haplotypes and pharmacokinetics/pharmacodynamics of irinotecan were investigated to identify pharmacogenetic markers.**

Methods **Associations between *UGT1A* haplotypes and the area under concentration curve ratio (SN-38 glucuronide/SN-38) or toxicities were analyzed in 177 Japanese cancer patients treated with irinotecan as a single agent or in combination chemotherapy. For association analysis, diplotypes of *UGT1A* gene segments [(*1A1*, *1A7*, *1A9*, *1A10*), and Block C (common exons 2–5)] and combinatorial haplotypes (*1A9-1A7-1A1*) were used. The relationship between diplotypes and toxicities was investigated in 55 patients treated with irinotecan as a single agent.**

Results **Among diplotypes of *UGT1A* genes, patients with the haplotypes harboring *UGT1A1*6* or **28* had significantly reduced area under concentration curve ratios, with the effects of *UGT1A1*6* or **28* being of a similar scale. A gene dose effect on the area under concentration curve ratio was observed for the number of haplotypes containing **28* or **6* (5.55, 3.62, and 2.07 for 0, 1, and 2 haplotypes, respectively, $P<0.0001$). In multivariate analysis, the homozygotes and double heterozygotes of **6* and **28* (**6/*6*, **28/*28* and **6/*28*) were significantly associated with severe neutropenia in 53 patients who received irinotecan monotherapy.**

Conclusions **The haplotypes significantly associated with reduced area under concentration curve ratios and neutropenia contained *UGT1A1*6* or **28*, and both of them should be genotyped before irinotecan is given to Japanese and probably other Asian patients. *Pharmacogenetics and Genomics* 17:497–504 © 2007 Lippincott Williams & Wilkins.**

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Introduction

Irinotecan, an anticancer prodrug, is widely applied for colorectal, lung, stomach, ovarian, and other various cancers. It is activated by carboxylesterases to SN-38 (7-ethyl-10-hydroxycamptothecin), which shows antitumor activity by inhibiting topoisomerase I [1,2]. SN-38 is subsequently glucuronidated by uridine diphosphate glucuronosyltransferases (*UGTs*) to form an inactive metabolite, SN-38 glucuronide (SN-38G) [3]. Dose-limiting toxicities of irinotecan are diarrhea and leukopenia [4], and reduced activity for SN-38G formation is closely related to severe toxicities [5]. Among UGT isoforms, UGT1A1 is abundant in both the liver and intestine and is thought to be mainly responsible for inactivation of SN-38 [3,6]. Genetic polymorphisms of *UGT1A1* result in reduced enzyme activity and increased toxicity by irinotecan. A significant association of *UGT 1A1*28*, a repeat polymorphism of the TATA box (-40_-39insTA) [3,7], with severe irinotecan-induced diarrhea/leukopenia was first reported in a retrospective study of Japanese cancer patients [8]. Subsequent pharmacogenetic studies in Caucasians have shown close associations of **28* with reduced glucuronidation of SN-38 and/or severe neutropenia/diarrhea [9–12]. These

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studies have clearly indicated that **28* is a good genetic marker for individualized irinotecan therapy. On the basis of these observations, the Food and Drug Administration of the United States has approved an amendment of the label for Camptosar (irinotecan HCl) and added a warning to consider a reduction in the starting dose of irinotecan for **28* homozygous patients (NDA 20-571/S-024/S-027/S-028).

There is significant racial difference in *UGT1A1* polymorphisms among Asians, Caucasians, and Africans [13]. Although the association of *UGT 1A1*28* with toxicities by irinotecan was first described in Japanese patients, its frequency in Japanese is one-third of that in Caucasians. Another low-activity allele **6* [211G > A(G71R)], which is not detected in Caucasians or Africans, is as frequent as the **28* allele in Japanese. Moreover, the area under concentration curve (AUC) ratio of SN-38G to SN-38 was decreased in patients having **6* haplotypes [14].

In addition to UGT1A1, recent studies have suggested possible contributions to SN-38G formation by UGT1A7, 1A9, and 1A10 [15–17], which are expressed in the gastrointestinal tract, the liver and intestine, and extrahepatic tissues, respectively [18]. Altered activity resulted from genetic polymorphisms of these isoforms, including *1A7*3* [387T > G(N129K), 391C > A(R131K), 622T > C(W208R)], *1A9*22* (-126_-118T_9 > T_{10}), *1A9*5* [766G > A(D256N)], and *UGT1A10*3* [605C > T(T202I)], but clinical relevance of these polymorphisms is yet to be elucidated [16,19–24]. Moreover, close linkages among *1A9*, *1A7*, and *1A1* polymorphisms were found in Caucasians and Asians in an ethnic-specific manner [20,25–27]. Therefore, comprehensive investigation that covers these genes, along with linkages among the polymorphisms, is needed, in each ethnic population, to evaluate associations between the genetic polymorphisms and pharmacokinetics, as well as clinical outcomes of irinotecan therapy.

Recently, we have analyzed the segmental and block haplotypes of *1A8*, *1A10*, *1A9*, *1A7*, *1A6*, *1A4*, *1A3* and *1A1*, and the common exons 2–5 (Block C) in a Japanese population, including the 177 cancer patients treated with irinotecan, and showed close linkages between the haplotypes, that is, *1A9*22* and *1A7*1*, *1A7*3* and *1A1*6*, and *1A7*3* and *1A1*28* [28]. Preliminary results of *UGT1A1* pharmacogenetics on 85 of these cancer patients were reported previously [14]. In the current study, we investigated the pharmacogenetics of irinotecan, focusing on diplotypes of the *UGT1A* complex covering *1A1*, *1A7*, *1A9*, *1A10*, and Block C (exons 2–5) of 177 patients, so as to elucidate haplotypes or genetic markers associated with altered glucuronidation of SN-38 and toxicities.

Methods

Patients and treatment schedule

Patients with cancers who started chemotherapy with irinotecan at two National Cancer Center Hospitals (Tokyo and Kashiwa, Japan) were eligible if they had not received irinotecan previously. Other eligibility criteria included bilirubin $\leq$ 2 mg/dl, aspartate aminotransferase (GOT) $\leq$ 105 IU/l, alanine aminotransferase (GPT) $\leq$ 120 IU/l, creatinine $\leq$ 1.5 mg/dl, white blood cell count $\geq$ 3000/μl, performance status of 0–2, and at least 4 weeks after the last chemotherapy (2 weeks for radiotherapy). Exclusion criteria were diarrhea, active infection, intestinal paralysis or obstruction, and interstitial pneumonitis. The ethics committees of the National Cancer Center and the National Institute of Health Sciences approved this study, and written informed consent was obtained from all participants.

Irinotecan was administered as a single agent or in combination chemotherapy at the discretion of attending physicians. Doses and schedules were according to approved usage in Japan; intravenous 90-min infusion at a dose of 100 mg/m^2 weekly or 150 mg/m^2 biweekly. In terms of combination chemotherapy, the dose of irinotecan was reduced according to clinical protocols.

Genetic polymorphisms of UGT1As and pharmacokinetics

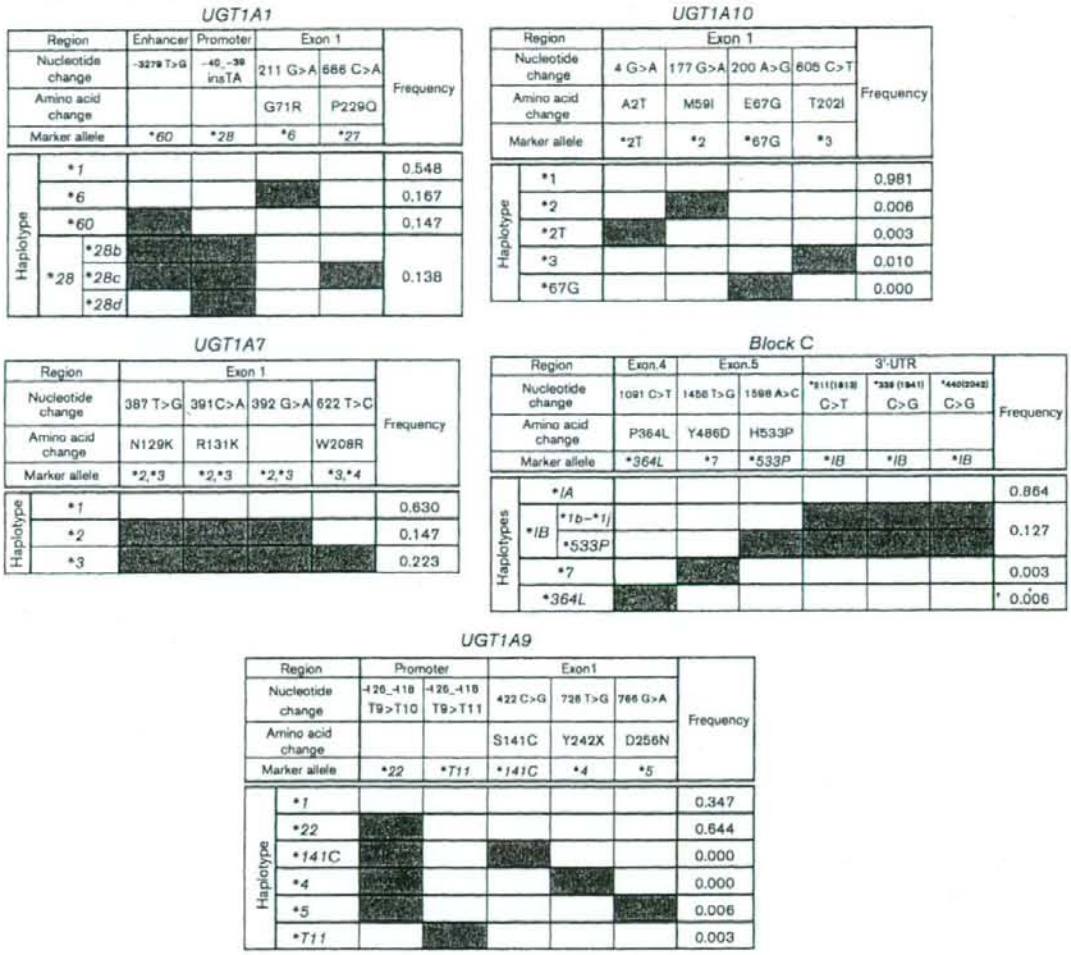
Detailed assay methods for genotypes of the *UGT1A* gene complex were reported previously [14,28]. In this study, we focused on the genetic variations in *UGT1A1*, *1A7*, *1A9*, and *1A10* and common exons 2–5, as they have been reported to contribute to the SN-38 glucuronidation. Haplotype analysis covering these regions was performed in our previous study [28], and haplotypes of each *UGT1A* segment [exon 1 for *1A1*, *1A7*, *1A9*, or *1A10*; and Block C (common exons 2–5)] are summarized in Fig. 1.

Pharmacokinetic analysis for irinotecan was performed as described previously [14]. Briefly, heparinized blood was collected before administration of irinotecan, as well as 0 and 20 min, and 1, 2, 4, 8, and 24 h after termination of the first infusion of irinotecan. Plasma concentrations of irinotecan, SN-38 and SN-38G were determined by the high-performance liquid chromatography [29], and AUC was calculated by the trapezoidal method using WinNonlin version 4.01 (Pharsight Corporation, Mountain View, California, USA). Associations between genotypes and the AUC ratio (AUC of SN-38G/AUC of SN-38) were evaluated in 176 patients.

Monitoring and toxicities

A complete medical history and data on physical examinations were recorded before the irinotecan therapy. Complete blood cell counts with differentials and platelet counts, as well as blood chemistry, were measured once a week during the first 2 months of irinotecan treatment. Toxicities were graded according to the Common Toxicity Criteria of National Cancer Institute version 2. Association of genetic factors with irinotecan toxicities was analyzed primarily in patients who received irinotecan as a single agent.

Fig. 1



Haplotypes of *UGT1A* gene segments (*UGT1A1*, *1A7*, *1A9*, *1A10*, and Block C) in 177 Japanese cancer patients. The tagging variations and haplotypes are shown. Variant alleles are indicated in grey. Definition of Block C haplotypes in our previous paper ([14]) (corresponding to Block 2) were slightly modified.

Statistical analysis

Statistical analysis on the differences in the AUC ratios (SN-38G/SN-38) among *UGT1A* genotypes was performed using the Kruskal–Wallis test, followed by nonparametric Dunnett's multiple comparison test, or with Wilcoxon test. Analysis of a gene–dose effect of each haplotype was performed using the Jonckheere–Terpestra test in the SAS system, version 5.0 (SAS Institute, Cary, North Carolina, USA). Relationship of *UGT1A* genetic polymorphisms to the toxicities of irinotecan was assessed by the χ^2 test via the use of using Prism version 4.0 (GraphPad Prism Software, San Diego, California, USA). The *P*-value of 0.05 (two-tailed) was set as a significant level, and the multiplicity adjustment was conducted for pharmacokinetics data with the false discovery rate [30].

To identify factors associated with the log-transformed AUC ratio of SN-38G/SN-38, multiple regression analysis was performed using age, sex, body surface area, dosage of irinotecan, history of smoking or drinking, performance status, coadministered drugs, serum biochemistry parameters at baseline, and *1A9-1A7-1A1* and Block C haplotypes (five or more chromosome numbers) or '*1A1*6* or **28*'. For multiple regression analysis of neutropenia, variables included the absolute neutrophil count at baseline and the dosing interval, in addition to

the other patient background factors described above. The multivariate analyses were performed by using JMP version 6.0.0 software (SAS Institute). The variables in the final models for both AUC ratio and neutropenia were chosen by forward and backward stepwise procedures at significance levels of 0.25 and 0.05, respectively.

Results

Patients and UGT1A haplotypes

Patient demographics and information on the treatment are summarized in Table 1. In addition to UGT1A1, UGT1A7, 1A9, and 1A10 were also reported to glucuronidate SN-38 [15–17]. In our previous study, haplotype analysis covering the *1A9* to *1A1* (5′–3′) gene segments was conducted, and the combinatorial diplotypes (*1A9-1A7-1A1*) of the patients were determined. It must be noted that close linkages between *1A9*22* and *1A7*1*, between *1A7*2* and *1A1*60*, and between *1A7*3* and *1A1*6* or *1A1*28* were observed as described previously [28]. To clarify the linkages between these segmental haplotypes (*1A9*, *1A7*, and *1A1*), we grouped the combinatorial (*1A9-1A7-1A1*) haplotypes into four categories (A–D) based on the *1A1* haplotypes (**1*, **6*, **60*, and **28*). Each group was further divided into the subgroups based on the previously defined Block 9/6 (including *1A9*, *1A7*, and *1A6)* haplotypes (Table 2). The frequency of Group B haplotypes (*B1-B4*) harboring *1A1*6* was 0.167 and higher than that of Group D haplotypes (*D1-D6*) with **28* (0.138) in this population.

Association of 1A9-1A7-1A1 diplotypes to SN-38G formation

When relationship between the *UGT1A* diplotypes (*1A9-1A7-1A1*) and the SN-38G/SN-38 AUC ratio was analyzed in the 176 cancer patients the AUC ratio for the diplotypes of *B2/B2*, *D2/A1*, and *D1/B2* was statistically significantly lower than the *A1/A1* diplotype (Fig. 2). These diplotypes harbored *1A1*6*, **28* or both. Significant gene–dose effects of *B2* (among *A1/A1*, *B2/A1*, and *B2/B2*) and *C3* (among *A1/A1*, *C3/A1*, and *C3/C3*) were also observed (Fig. 2). As no significant differences in AUC ratios were observed between *D1/A1* and *D2/A1*, *D1/C3* and *D2/C3*, and *D1/B2* and *D2/B2*, the haplotype combination *1A9*1-1A7*3* or *1A9*22-1A7*1* was not influential on the AUC ratio.

As the effect of diplotypes harboring *UGT1A1* polymorphism was prominent, we grouped the whole gene (*1A9-1A7-1A1*) diplotypes according to the *1A1* diplotypes (the upper part of Fig. 2). Patients with **6* or **28* (except for **28/*28*) haplotypes had significantly lower AUC ratios than the wild-type (**1/*1*), and significant gene–dose effects were observed for **28* (among **1/*1*, **28/*1*, and **28/*28*) and **6* (among **1/*1*, **6/*1* and **6/*6*). A significant additive effect of **6* and **28* on the decreased AUC ratio was also observed when the values for **28/*1* were compared with those for **28/*6* (Fig. 2 and Table 3).

Regarding other polymorphisms, a statistically nonsignificant tendency to decrease the AUC ratio was observed for **60*

Table 1 Characteristics of Japanese cancer patients in this study

		No. of participants	
Age			
Mean/range	60.5/26–78	177	
Sex			
Male/female		135/42	
Performance status	0/1/2	84/89/4	
Combination therapy and tumor type (initial dose of irinotecan; mg/m^2)			
Irinotecan monotherapy	Lung (100)	21	
	Colon (150)	28	
	Others (100)	7	
With platinum-containing drug[a]	Lung (60)	58[b]	48 [60][c]
	Stomach (70)	9	9 [80][c]
	Others (60)	5	5 [80][c]
With 5-fluorouracil (including tegafur)	Colon (100 or 150)	34	
	Others (90 or 100)	2	
With mitomycin-C	Stomach (150)	10	
	Colon (150)	1	
With amrubicin	Lung (60)	2	
Previous treatment			
Surgery	Yes/no	85/92	
Chemotherapy	Yes/no	97/80	
Radiotherapy	Yes/no	26/151	
Smoking history	Yes/no	29/148	

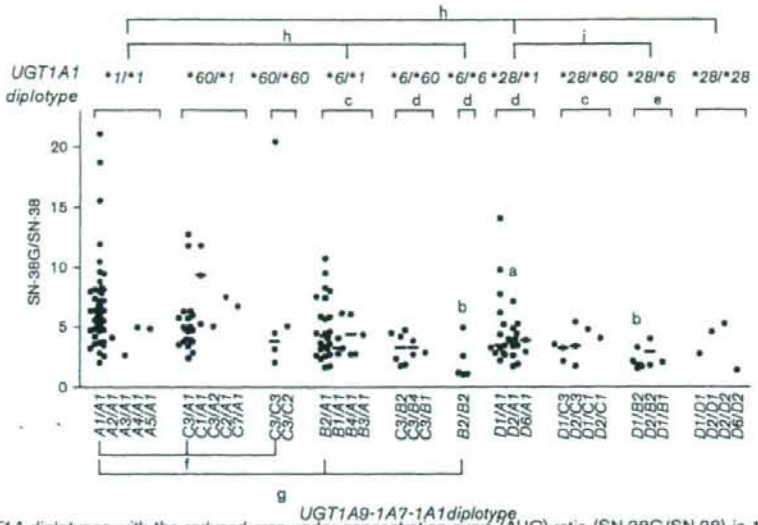
[a]Cisplatin, cisplatin plus etoposide or carboplatta.
[b]Two and eight patients received cisplatin and etoposide and carboplatin, respectively.
[c]Number of cisplatin-administered patients [initial dose of cinlatin (mg/m^2) is shown in brackets].

Table 2 Combinatorial haplotypes covering *UGT1A9*, *UGT1A7*, and *UGT1A1*

	Block haplotype[a]			Combination of segmental haplotypes	Cancer patients	
Haplotype	Block 9/6	Block 4	Block 3/1	1A9-1A7-1A1	*N*[b]	Frequency
A1[c]	*I	*1	*I	*22-*1-*1	189	0.534
	*I	*3	*I			
A3	*III	*1	*I	*1-*2-*1	2	0.006
A2	*II	*1	*I	*1-*3-*1	1	0.003
A4	*IV	*1	*I	*22-*3-*1	1	0.003
A5				*T11-*1-*1	1	0.003
B2[c]	*II	*1	*III			
	*II	*1	*VI	*1-*3-*6	47	0.133
	*II	*4	*VI			
B4	*IV	*1	*III	*22-*3-*6	6	0.017
B1	*I	*1	*III	*22-*1-*6	5	0.014
	*I	*1	*VI			
B3	*III	*1	*III	*1-*2-*6	1	0.003
C3[c]	*III	*3	*IV			
	*III	*1	*IV			
	*III	*3	*V	*1-*2-*60	44	0.124
	*III	*1	*V			
C1	*I	*3	*IV	*22-*1-*60	5	0.014
	*I	*1	*IV			
C2	*II	*3	*IV	*1-*3-*60	2	0.006
C7	*VII	*3	*V	*22-*2-*60	1	0.003
D1	*I	*1	*IIa	*22-*1-*28	23	0.065
	*I	*1	*IIc			
D2	*II	*1	*IIa			
	*II	*3	*IIa	*1-*3-*28	22	0.062
	*II	*1	*IIc			
D6	*VI	*1	*IIb	*1-*2-*28	4	0.011
				Total	354	1.000

[a]Block haplotypes described in Ref. [28] are shown for reference. *1A9* and *1A7* are included in block 9/6 and *1A1* is included in block 3/1.
[b]Number of chromosomes.
[c]Major combinatrial haplotypes.

Fig. 2



The association of *UGT1A* diplotypes with the reduced area under concentration curve (AUC) ratio (SN-38G/SN-38) in 176 Japanese cancer patients who received irinotecan. The whole gene (*1A9-1A7-1A1*) diplotypes are shown below the abscissa and the *UGT1A1* diplotypes are indicated in the upper part of the figure. Each point represents a patient value, and the median is indicated by a bar. Significant reductions in the AUC ratio were detected in the *B2/B2*, *D2/A1*, and *D1/B2* compared with *A1/A1* for the whole gene diplotypes [Kruskal–Wallis test ($P=0.0009$) followed by Dunnett's multiple comparison test]. As for the *1A1* diplotypes, significant reductions were detected in the **6/*1*, **6/*60*, **6/*6*, **28/*1*, **28/*60*, and **28/*6* compared with the **1/*1* group [Kruskal–Wallis test ($P<0.0001$) followed by Dunnett's multiple comparison test]. Gene–dose effects on the reduced AUC ratio were significant for **6* and **28* (Jonckheere–Terpestra test). A significant additive effect of **6* on the reduced AUC ratio by **28* was detected by comparing **28/*1* and **28/*6*. $^{a}P<0.05$ and $^{b}P<0.01$ against *A1/A1* group (Dunnett's multiple comparison test); $^{c}P<0.05$, $^{d}P<0.01$, and $^{e}P\&<0.001$ against the **1/*1* group (Dunnett's multiple comparison test); $^{f}P<0.05$, $^{g}P<0.001$, and $^{h}P<0.0001$ (Jonckheere–Terpestra test for gene–dose effect); $^{i}P<0.01$ (Wilcoxon test).

($P = 0.1134$). No significant effects on the AUC ratio were observed for Block C (exon 2–5) haplotypes or rare variations including *1A10* (**2T*, **2*, or **3*) and *1A9* (**5*, **T11*).

Multiple regression analysis of the area under concentration curve ratio

We further assessed the impact of *UGT1A* genetic factors on the AUC ratio by multiple regression analysis. First, we used the *1A9-1A7-1A1* and Block C haplotypes as genetic factors. The AUC ratio was significantly associated with the haplotypes *B2*, *D1*, and *D2* and serum biochemistry parameters indicating hepatic or renal function before treatment. The Groups B and D haplotypes harbor *1A1*6* and **28*, respectively. The dependency on specific *1A7* or *1A9* polymorphisms, however, was not obtained, considering the contributions of both *D1* and *D2*. As *1A1*6* and **28* are mutually exclusive and their effects are comparable, we grouped *1A1*6* and **28* into the same category in the final multiple regression model (Table 4). The final model confirmed the significant contribution of this genetic marker (**6* or **28*) to the AUC ratio.

Effects of the genetic marker '*6 or *28' on pharmaco kinetic parameters

Then, a dose effect of the genetic marker '**6* or **28*' on pharmacokinetic parameters was further analyzed

Table 3 AUC ratio of SN-38 glucuronide to SN-38 for *UGT1A1* diplotypes

Diplotype	Number of patients	AUC ratio		*P*-value* (vs. **1/*1*)
		Median	Interquartile range	
**1/*1*	55	6.13	4.72–7.79	
**1/*60*	25	5.04	3.85–6.52	0.9803
**60/*60*	5	4.48	2.57–12.74	0.8141
**6/*1*	32	4.03	2.74–5.97	0.0126
**6/*60*	9	2.84	2.09–4.33	0.0021
**6/*6*	5	1.19	1.06–3.74	0.0012
**28/*1*	26	3.65	2.76–5.21	0.0040
**28/*60*	8	3.44	2.68–4.40	0.0261
**28/*6*	7	2.03	1.65–3.26	<0.0001
**28/*28*	4	3.65	2.05–4.92	0.2322

AUC, area under concentration curve.
*Dunnett's multiple comparison test.

(Fig. 3). Patients with one haplotype harboring either **6* or **28* (**6/*1*, **6/*60*, **28/*1*, and **28/*60*) had lower SN-38G/SN-38 AUC ratios (median, 3.62; interquartile range, 2.74–5.18) than patients without **6* or **28* (**1/*1*, **60/*1*, and **60/*60*) (5.55, 4.13–7.26), and patients with two haplotypes harboring **6* or **28* (**6/*6*, **28/*28*, and **28/*6*) had the lowest AUC ratio (2.07, 1.45–3.62) ($P < 0.0001$, Fig. 3a). Similarly, the number of the **6* or **28*-containing haplotypes affected the AUC ratios of SN-38 to irinotecan (Fig. 3b). When the correlations

between irinotecan dosage and the AUC of SN-38 were tested, different correlations were obtained according to the number of the haplotypes (Fig. 3c). The slope of regression line for one and two haplotypes harboring **6* or **28* was 1.4-fold and 2.4-fold greater, respectively, than that for the diplotype without **6* or **28*.

Associations of UGT1A1 genetic polymorphisms with toxicities

Association between genetic polymorphisms and toxicities was investigated in patients receiving irinotecan as a single agent. One patient was referred to another hospital 3 days after the first administration of irinotecan without evaluating toxicities and was lost in terms of follow-up. Therefore, association between genetic polymorphisms and toxicities was investigated in 55 patients. Six (11%) and 14 (25%) patients experienced grade 3 or greater diarrhea and neutropenia, respectively. As for the *1A9-1A7-1A1* diplotypes, a higher incidence of grade 3 or greater neutropenia was observed in *D1/B2* (*1A1*28/*6*) (100%, $n = 3$) than in *A1/A1* (11.8%, $n = 17$) ($P = 0.0088$, Fisher's exact test), indicating clinical impact of the genetic marker *1A1*6* or **28*. As for the dose effect of '**6 or *28*', incidences of grade 3 or 4 neutropenia were 14, 24, and 80% for 0, 1, and 2 haplotypes harboring these markers, respectively (Table 5). A significant association between '**6* or **28*'and neutropenia was also observed for 62 patients who received irinotecan in combination with cisplatin (Table 5). No association, however, was observed between diarrhea and the marker '**6* or **28*'.

Multivariate analysis for irinotecan toxicities

We further evaluated the effect of the genetic marker '**6* or **28*' on neutropenia in multivariate analysis, and confirmed a significant correlation of '**6* or **28*' with the nadir of absolute neutrophil counts (Table 6). Elevated alkaline phosphatase levels and the absolute neutrophil count at baseline were also significant.

Discussion

The association study with the *1A9-1A7-1A1* diplotypes revealed that the reduction in inactivation of SN-38, as well as neutropenia, was dependent on the Groups B and D haplotypes which corresponded to the *1A1*6* and **28* segmental haplotypes. Also, multivariate analyses clearly showed clinical significance of the genetic marker '**6* or **28*' for both pharmacokinetics and toxicity of irinotecan in Japanese patients (Tables 3 and 6). *UGT1A1*6* and **28* were mutually exclusive [14] and contributed to the reduction in glucuronidation of SN-38 to the same extent. Therefore, the activity of SN-38 glucuronidation in individuals depended on the number of the haplotypes harboring **6* or **28*. Although the role of *1A1*28* for irinotecan toxicity has been focused on [8–12], this study strongly suggests that **6* should be tested in addition to **28* before starting chemotherapy with irinotecan in Japanese patients.

The clinical importance of **6* for neutropenia by irinotecan was also supported by a recent report in Korean patients who received irinotecan and cisplatin [31]. Although no patients with irinotecan as a single agent were homozygous for **6* in our study, clinical significance of the double heterozygote, **6/*28*, was clearly demonstrated. Among patients treated with irinotecan in combination chemotherapy, the majority of patients received platinum agents in our study. A significant association of '**6* or **28*' with a higher incidence of grade 3 or 4 neutropenia was also observed in patients who received irinotecan and cisplatin (Table 5). These findings further support the necessity of testing '**6* or **28*' before irinotecan is given to patients.

As possible enhancement of toxicities by the **27* allele was suggested [8], we evaluated the effect of the **28c* haplotype, which had an additional single-nucleotide polymorphism [**27*; 686C > A(P229Q)] to the **28* allele (-40_-39insTA). In our cohort of patients, there were three **28c* heterozygotes (**28c/*1*) and one double heterozygote (**28b/*28c*). The values of the AUC ratio were within the range of variations of the **28* group, and no additional impact of **28c* was observed in relation to toxicities.

Although the decreasing trend of the AUC ratio for *1A1*60* (and combinatorial haplotype *C3*) was observed (Fig. 2), the contribution of *1A1*60* to toxicities was not clearly demonstrated in this study as reported in the Japanese retrospective study [32].

In addition to UGT1A1, recent studies have suggested possible contributions of UGT1A7, 1A9, and 1A10 to SN-38G formation [15–17]. An in-vitro study demonstrated that *1A7*3* [387T > G(N129K), 391C > A(R131K), 622T > C(W208R)] had reduced activity in terms of SN-38G formation [16]. Results of clinical studies, however, on the association between *1A7* polymorphisms and irinotecan toxicity/efficacy are inconsistent, whereas different populations with different combination therapies were used [19,20]. Furthermore, it was reported that the *UGT1A7* polymorphisms (**2* and **3*), which were linked to *1A9*1*, were associated with a lowered incidence

Table 4 Multiple regression analysis toward the AUC ratio (SN-38G/SN-38)[a]

Variable	Coefficient	F-value	*P*-value	R^2	Intercept	*N*
				0.410	0.8869	176
**6* or **28*	−0.189	70.2	<0.0001			
Age	0.005	8.88	0.0033			
Serum albumin level[b]	−0.136	9.92	0.0019			
Serum GOT and ALP[c]	0.070	8.88	0.0033			
Serum creatinine[d]	0.210	7.23	0.0079			

ALP, alkaline phosphatase; AUC, area under concentration curve.
[a]The values after logarithmic conversion were used as an objective variable.
[b]The absolute value (g/dl) before irinotecan treatment.
[c]Grade 1 or greater scores in both serum GOT and ALP before irinotecan treatment.
[d]Grade 1 or greater scores in serum creatinine before irinotecan treatment.