Fig. 1. Kaplan-Meier plot of progression-free survival (adenocarcinoma versus nonadenocarcinoma histology).
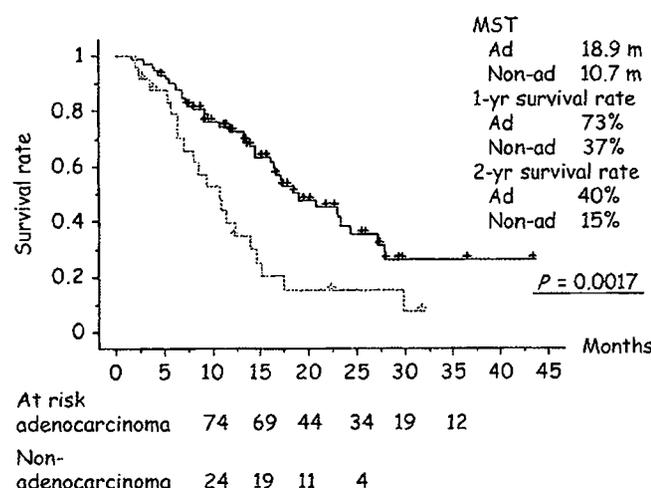

Fig. 3. Kaplan-Meier plot of overall survival (adenocarcinoma versus nonadenocarcinoma histology).

Fig. 2. Kaplan-Meier plot of progression-free survival (smoker versus non-smoker).
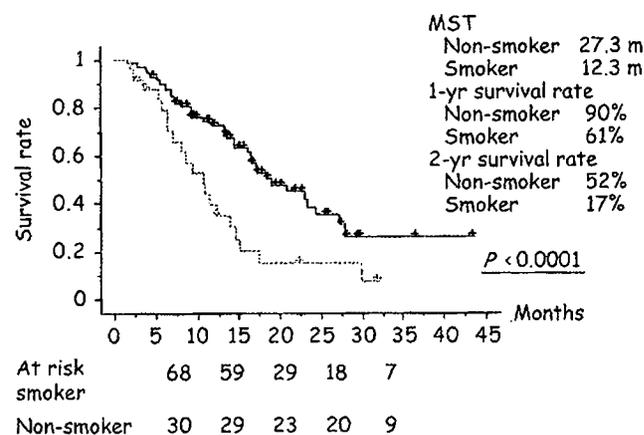
Fig. 4. Kaplan-Meier plot of overall survival (smoker versus non-smoker).

and between non-smokers versus smokers ($P < 0.0001$). Of particular note, median OS of smokers was 12.3 months, and non-smokers was 27.3 months. Two-year survival rates were 17% and 52% in smokers and non-smokers, respectively (Figs 3,4).

To identify factors influencing OS in patients who received second-line therapy ($n = 76$), multivariate analysis was performed with covariates including histology (adenocarcinoma versus other), smoking history (non-smoker versus smoker), PS (0 versus 1), docetaxel (use versus non-use) and EGFR-TKI (use versus non-use). The use of EGFR-TKI was identified as a significant prognostic factor associated with longer OS, together with non-smoking history and PS 0. The use of docetaxel was not associated with an increase in OS in this study (Table 4). When interaction terms between clinical variables and EGFR-TKI treatment were included in the model, no significant interaction was detected ($P = 0.354$ and 0.515 for smoking history × EGFR-TKI and histology × EGFR-TKI, respectively). In the exploratory Cox analysis, prognostic advantage for non-smoking history and adnocarcinoma histology was more prominent in patients who received EGFR-TKI treatment after adjustment for PS, suggesting a potential interaction between these favorable clinical variables and EGFR-TKI treatment. Compared with smokers, hazard ratio
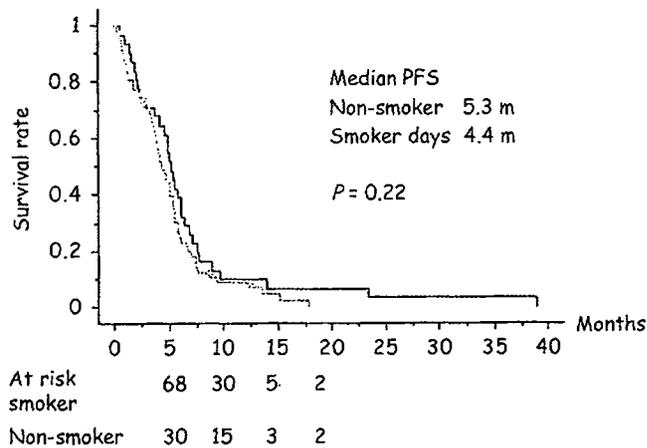
Table 4. Cox regression analysis of prognostic factors for overall survival after second-line treatment: a stepwise forward procedure

Factor	Variable	P-value HR (95% CI)
Histology	Adenocarcinoma versus other	0.0639 —
Smoking	Non-smoker versus smoker	0.0052 0.325 (0.148–0.715)
PS	0 versus 1	0.0258 (0.258–0.917)
Docetaxel	– versus +	0.6720 —
EGFR-TKI	– versus +	0.0084 2.844 (1.306–1.823)

PS, performance status; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; HR, hazard ratio; CI, confidence interval.

of non-smokers with or without EGFR-TKI was 0.961 (95% CI, 0.209–4.420) and 0.193 (0.083–0.449), respectively. Likewise, the hazard ratio of adenocarcinoma patients with or without EGFR-TKI was 0.429 (0.138–1.334) and 0.387 (0.187–0.800), respectively, compared with patients with other histologies.

Table 5. Historical comparison of outcomes in our study and the FACS study[2]

	FACS[1] (n = 145)	This study (n = 98)
Response rate (%)	32	20
Median PFS (months)	4.5	4.8
Median OS (months)	12.3	16.5
1-year survival rate (%)	51	64
Second-line therapy, n (%)	87 (60)	76 (78)
Docetaxel	25 (17)	42 (43)
EGFR-TKI	9 (6)	29† (30)
Other	58 (40)	5 (5)

†25 patients were treated with Gefitinib. FACS, Four-Arm Cooperative Study; PFS, progression-free survival; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor.

Discussion

Since the results of a large meta-analysis revealed that platinum-based chemotherapy prolonged OS of patients with advanced NSCLC compared with best supportive care (BSC)[8] this therapy has been considered standard first-line treatment for advanced NSCLC worldwide. Median OS with carboplatin/paclitaxel – the most commonly used standard therapy outside Japan – has been reported to be 8–14 months[9–13] similar to the median OS (12.3 months) observed in the FACS trial conducted in Japan.[2,3]

Comparison of outcomes following carboplatin/paclitaxel treatment in our study with the results obtained with the same regimen in the FACS study, showed there was little difference in median PFS (4.8 versus 4.5 months), but median OS was approximately 4 months longer at our hospital (16.5 versus 12.3 months). With the recent approval of EGFR-TKIs, the use of these agents as second-line chemotherapy has increased since the FACS study was performed (30% of patients in this study versus 6% of patients in the FACS study) (Table 5). This observation suggests that the better treatment outcomes obtained in our study compared with those of FACS may be attributable to the effect of anticancer agents used in second-line and subsequent treatment, especially EFGR-TKI (gefitinib was used in most cases). In fact, the result of subgroup analysis by patient demographics in our study demonstrated a marked prolongation of OS for non-smokers and patients with adenocarcinoma, both of which are known to be factors associated with high responsiveness to EGFR-TKI. Furthermore, the multivariate analysis in patients receiving second-line treatment, revealed that EGFR-TKI use was an independent prognostic factor.

Generally, the prolongation of OS is the ultimate goal of anticancer therapy and an important clinical outcome in the evaluation of the effect of first-line treatment for NSCLC. With the emergence of potent anticancer agents in the second-line setting, therapy administered after the occurrence of progressive disease becomes a confounding factor in the interpretation of

OS. To overcome this issue of confounding, there may be value in using prolongation of PFS as the primary outcome of first-line trials. Currently, the Food and Drug Administration (FDA) requires an applicant to demonstrate prolonged survival as an approval condition for new anticancer agents.[14] However, the European Agency for Evaluation of Medical Products (EMEA) has accepted PFS as the primary endpoint in some instances, and our present study result supports this view.[15]

The results of the BR21 trial showed that erlotinib significantly prolonged OS compared with placebo (6.7 versus 4.7 months, hazard ratio [HR] = 0.70). In the multivariate analysis, Asian origin (P = 0.01), adenocarcinoma histology (P = 0.004) and non-smoking status (P = 0.048) correlated with prolonged OS.[6] In the preplanned subgroup analysis in the ISEL trial, significantly longer survival was seen with gefitinib compared with placebo in patients of Asian origin (9.5 versus 5.5 months, HR = 0.66) and never-smokers (8.9 versus 6.1 months, HR = 0.67).[7] Although these two studies did not include Japanese patients, the findings might be extrapolated into Japanese populations. Since the reports of Paez et al.[16] and Lynch et al.[17] in April and May 2004, respectively, numerous studies of EGFR mutations have been conducted in a short period and studies conducted in Japan have reported a good correlation between OS and EGFR mutations in patients treated with gefitinib.[18–20] Moreover, the incidence of EGFR mutations is more frequent in women, patients with adenocarcinoma, never-smokers and Japanese patients[16,17] suggesting that there is a correlation between clinical and molecular factors and clinical benefit from EGFR-TKIs.

Although EGFR mutations are of interest as a biomarker that can be predictive of the effect of gefitinib, especially in patients of Asian or Japanese origin, their immediate clinical application for patient selection is not always possible, due to issues including method determination, cost and convenience. Correlation between response to gefitinib and EGFR copy number determined by fluorescence in situ hybridization (FISH) has attracted attention in the West as an alternative potential biomarker[21,22] and this needs to be further investigated in Japan. Acknowledging the need to pay close attention to future research trends, we believe further discussion into how to select those patient populations most likely to benefit from gefitinib in routine clinical practice is required. It is important to establish whether patients could be selected on the basis of biomarker data such as EGFR mutations, or EGFR over-expression, or clinical characteristics such as histological subtype and smoking history. Nevertheless, selection of appropriate patients for EGFR-TKI therapy is undoubtedly necessary, and we hope that future research will be able to identify possible methods as soon as possible. Once identified these will require validation in large-scale prospective clinical studies.

In conclusion, this retrospective study demonstrated a marked prolongation of overall survival in patients with adenocarcinoma and non-smoking history who received carboplatin/paclitaxel as first-line treatment. Our study results suggest that the use of EGFR-TKI (especially gefitinib) after first-line treatment may be associated with an improvement in overall survival.

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

Hironobu Minami[a], Kimie Sai[b,c], Mayumi Saeki[b], Yoshiro Saito[b,d],
Shogo Ozawa[b,e], Kazuhiro Suzuki[c], Nahoko Kaniwa[b,f], Jun-ichi Sawada[b,d],
Tetsuya Hamaguchi[g], Noboru Yamamoto[g], Kuniaki Shirao[g], Yasuhide Yamada[g],
Hironobu Ohmatsu[h], Kaoru Kubota[h], Teruhiko Yoshida[i], Atsushi Ohtsu[j]
and Nagahiro Saijo[k]

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.

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 *UGT1A1*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

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 ≤ 2 mg/dl, aspartate aminotransferase (GOT) ≤ 105 IU/l, alanine aminotransferase (GPT) ≤ 120 IU/l, creatinine ≤ 1.5 mg/dl, white blood cell count ≥ 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

UGT1A1

Region	Enhancer	Promoter	Exon 1		Frequency
Nucleotide change	-3279 T>G	-40_-39 insTA	211 G>A	686 C>A	
Amino acid change			G71R	P229Q	
Marker allele	*60	*28	*6	*27	
Haplotype *1					0.548
*6					0.167
*60					0.147
*28 *28b / *28c / *28d					0.138

UGT1A10

Region	Exon 1				Frequency
Nucleotide change	4 G>A	177 G>A	200 A>G	605 C>T	
Amino acid change	A2T	M59I	E67G	T202I	
Marker allele	*2T	*2	*67G	*3	
Haplotype *1					0.981
*2					0.006
*2T					0.003
*3					0.010
*67G					0.000

UGT1A7

Region	Exon 1				Frequency
Nucleotide change	387 T>G	391C>A	392 G>A	622 T>C	
Amino acid change	N129K	R131K		W208R	
Marker allele	*2,*3	*2,*3	*2,*3	*3,*4	
Haplotype *1					0.630
*2					0.147
*3					0.223

Block C

Region	Exon.4	Exon.5		3'-UTR			Frequency
Nucleotide change	1091 C>T	1456 T>G	1598 A>C	*211(1813) C>T	*339(1941) C>G	*440(2042) C>G	
Amino acid change	P364L	Y486D	H533P				
Marker allele	*364L	*7	*533P	*IB	*IB	*IB	
Haplotypes *IA							0.864
*IB *1b-*1j / *533P							0.127
*7							0.003
*364L							0.006

UGT1A9

Region	Promoter		Exon1			Frequency
Nucleotide change	-126_-118 T9>T10	-126_-118 T9>T11	422 C>G	726 T>G	766 G>A	
Amino acid change			S141C	Y242X	D256N	
Marker allele	*22	*T11	*141C	*4	*5	
Haplotype *1						0.347
*22						0.644
*141C						0.000
*4						0.000
*5						0.006
*T11						0.003

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

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/m²)			
Irinotecan monotherapy	Lung (100)	21	
	Colon (150)	28	
	Others (100)	7	
With platinum-containing	Lung (60)	58[b]	48 [60][c]
drug[a]	Stomach (70)	9	9 [80][c]
	Others (60)	5	5 [80][c]
With 5-fluorouracil	Colon (100 or 150)	34	
(including tegafur)	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/m²) is shown in brackets].

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

Haplotype	Block haplotype[a]			Combination of segmental haplotypes	Cancer patients	
	Block 9/6	Block 4	Block 3/1	1A9-1A7-1A1	N[b]	Frequency
A1[c]	**1*	**1*	**1*	**22-*1-*1*	189	0.534
	*1	**3*	**1*			
A3	**III*	**1*	**1*	**1-*2-*1*	2	0.006
A2	**II*	**1*	**1*	**1-*3-*1*	1	0.003
A4	**IV*	**1*	**1*	**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	**1*	**1*	**III*	**22-*1-*6*	5	0.014
	*1	**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	**1*	**3*	**IV*	**22-*1-*60*	5	0.014
	*1	**1*	**IV*			
C2	**II*	**3*	**IV*	**1-*3-*60*	2	0.006
C7	**VII*	**3*	**V*	**22-*2-*60*	1	0.003
D1	**1*	**1*	**IIa*	**22-*1-*28*	23	0.065
	*1	**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
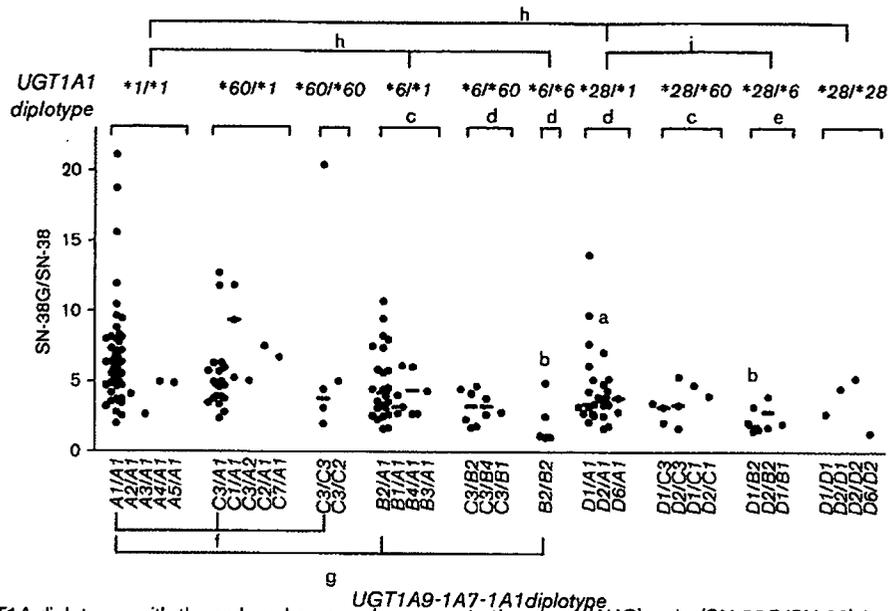
The association of *UGT1A* diplotypes with the reduced area under concentration curve (AUC) ratio (SN-38G/SN-38) in 176 Japanese cancer patients who received irinotecan. The whole gene (*1A9-1A7-1A1*) diplotypes are shown below the abscissa and the *UGT1A1* diplotypes are indicated in the upper part 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 *UGT1A* diplotypes

| Diplotype | Number of patients | AUC ratio | | P-value^a (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.
^{a}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

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

Variable	Coeffi-cient	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.

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

Fig. 3
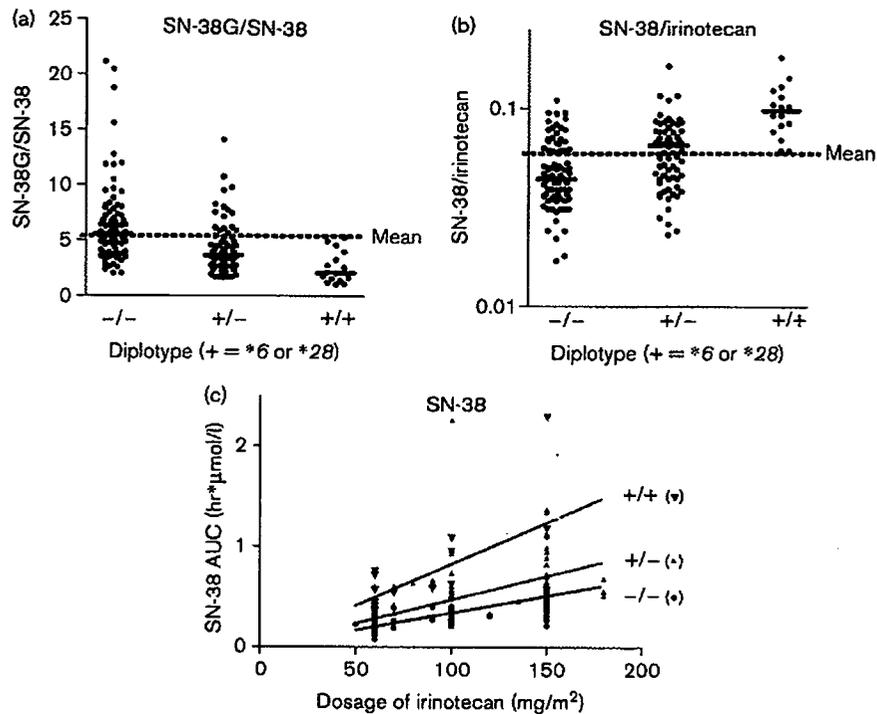
Effects of the genetic marker of UGT1A1 '*6 or *28' on the area under concentration curve (AUC) ratios of SN-38G/SN-38 (a) and SN-38/irinotecan (b), and SN-38 by irinotecan dosage (c) in 176 Japanese cancer patients after irinotecan treatment.

Table 5 Association of UGT1A1*6 and *28 with irinotecan toxicities

Diplotype (+ =*6 or *28)	Number of patients	Diarrhea (grade 3)	Neutropenia (grade 3 or 4)
Irinotecan monotherapy			
-/-	21	3 (14.3%)[a]	3 (14.3%)
+/-	29	2 (6.90%)	7 (24.1%)
+/+	5	1 (20.0%)	4 (80.0%)
P-value[b]		0.8500	0.0117
P-value[c]		0.3889	0.0124
With cisplatin			
-/-	35	1 (2.9%)	20 (57.1%)
+/-	20	2 (10.0%)	14 (70.0%)
+/+	7	1 (14.3%)	7 (100%)
P-value[b]		0.1747	0.0315
P-value[c]		0.3886	0.0863

[a]Percentage of the patient number in each diplotype is indicated in parentheses.
[b]Chi-squared test for trend.
[c]Fisher's exact test, (-/- and +/-) vs. +/+.

Table 6 Multiple regression analysis of the nadir of absolute neutrophil counts in the patients with irinotecan monotherapy

Variable	Coefficient	F-value	P-value	R^2	Intercept	N
				0.3942	643	53
Serum ALP[a]	-349.9	12.2	0.0010			
Neutrophil count before irinotecan treatment	0.2466	13.5	0.0006			
*6 or *28	-369.1	6.40	0.0146			

[a]Grade 1 or greater scores of serum ALP before irinotecan treatment.

of diarrhea in the irinotecan/capecitabine regimen, in which diarrhea was a major toxicity [20]. A highly frequent allele 1A9*22 with an insertion of T into the nine T repeats in the promoter region (-126_-118T$_9$ > T$_{10}$) was shown to have an enhanced promoter activity in an in-vitro reporter assay [21], whereas 1A9 protein expression levels did not change in the clinical samples [22]. Rare variations, 1A9*5 [766G > A(D256N)] and UGT1A10*3 [605C > T(T202I)], were shown to cause reduced activity *in vitro*, but their clinical importance is still unknown [23,24]. Moreover, close linkages among 1A9, 1A7, and 1A1

polymorphisms were found in Caucasians and Asians in an ethnic-specific manner [20,25–28].

Our study also revealed close linkages between 1A9*22 and 1A7*1, 1A7*3 and 1A1*6 or *28 [28]. This fact makes it difficult to draw firm conclusions about the effects of 1A7*3 and 1A9*22 themselves. It is, however, reasonable to conclude that the degree of neutropenia depends on the activity of UGT1A1, because UGT1A1 is a major UGT1A enzyme in the liver and plays a primary role for regulating plasma concentrations of SN-38.

Taken together, for practical application to individualized irinotecan therapy, genotyping of UGT1A1*6 and *28 would be beneficial and necessary in Japanese cancer patients to avoid severe adverse reactions. The frequency

of homozygotes for '*6 or *28' (namely, *6/*6, *6/*28, and *28/*28) is approximately 10%, which is comparable to the frequency of *28 homozygotes in Caucasian populations. In our study, it may be difficult to establish definite guidelines for dose reductions of irinotecan for patients homozygous for '*6 or *28'. Considering, however, 2.4-fold steep relationship between the dose of irinotecan and the AUC of SN-38 for patients homozygous for '*6 or *28' compared with patients without '*6 or *28' (Fig. 3c), the dose for patients homozygous for '*6 or *28' should be reduced to a half of the dosage recommended for other patients. Prospective studies are necessary to confirm the validity of the recommendation for dose reduction in Japanese cancer patients homozygous for '*6 or *28'.

Acknowledgements

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Metastatic Serous Adenocarcinoma Arising in the Adnexa Uteri and Forming Pleural Cysts on the Diaphragmatic Pleura

Kunimitsu Kawahara[1], Masashi Kobayashi[2], Shinji Sasada[2],
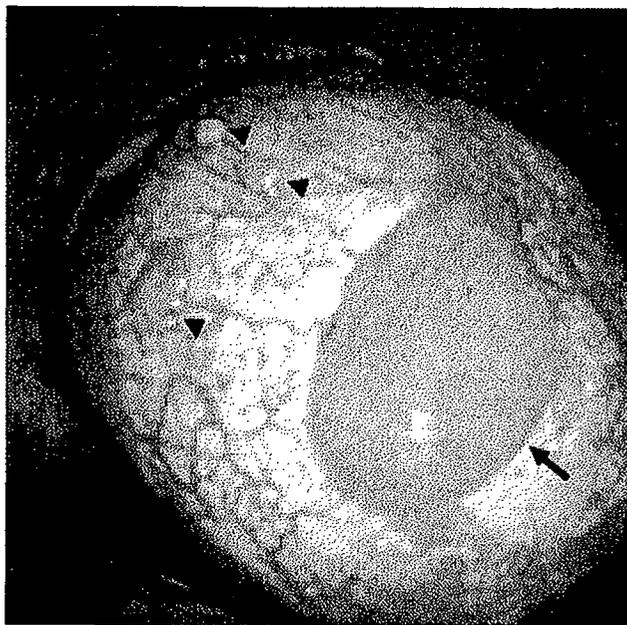Kaoru Matsui[2] and Teruo Iwasaki[3]

Figure 1. Left thoracoscopy showed a pleural cyst measuring 1.5cm in diameter (arrow) and adjacent daughter cysts (arrowheads) on the diaphragmatic pleura.

A 74-year-old woman consulted our hospital complaining of cough that had persisted for the previous 3 months. Chest computed tomographic (CT) scan showed bilateral pleural effusion without any pulmonary lesions. Pleural effusion cytology showed adenocarcinoma. Barium enema, gastroduodenoscopy and abdominal CT did not demonstrate any abnormal findings. Serum CEA, NSE and CYFRA21-1 were 26.8 (cutoff: 5) ng/ml, 43.7 (cutoff: 10) ng/ml and 67.5 (cutoff: 3.5) ng/ml, respectively. After removal of 1,500 ml of pleural effusion, left thoracoscopy showed a few eccentric pleural cysts on the diaphragmatic pleura (Fig. 1). No pleural nodule suggestive of malignancy was recognized. The content of the cyst was clearly serous fluid. Pathologic examination of the cyst showed a small focus of adenocarci-
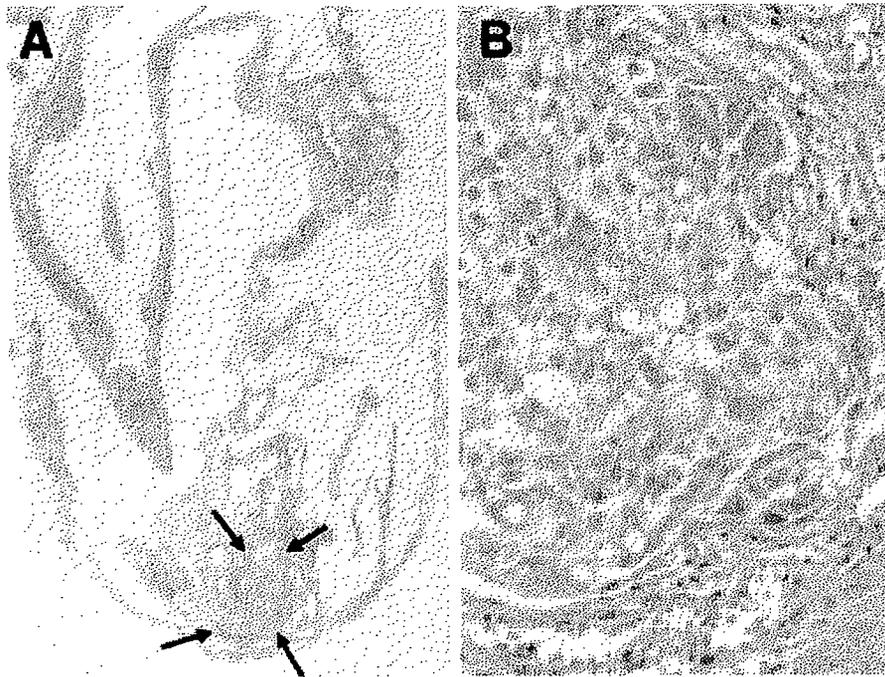
Figure 2. A: Microscopically, the pleural cyst was unilocular. A small focus of adenocarcinoma was recognized in the cyst wall (arrows). B: Most tumor cells had abundant clear or pale eosinophilic cytoplasm, oval nuclei and inconspicuous nucleoli. Stain: hematoxylin and eosin; magnification A: ×2.5, B: ×100.

noma (Fig. 2a, b). Immunohistochemical studies showed that these carcinoma cells were positive for AE1/AE3, EMA, CA125 and cytokeratin (CK)-7, but negative for CEA, TTF-1 and CK-20. The tentative diagnosis was Stage IV pulmonary adenocarcinoma. Systemic chemotherapy achieved stable disease. Six months later, the patient underwent surgery for right uterine adnexal tumor with diffuse peritoneal dissemination. Pathologic examination of the resected specimen demonstrated that the tumor was a poorly differentiated serous adenocarcinoma arising in the right adnexa uteri. Conclusively, we diagnosed pleural lesions as distant metastases of uterine adnexal serous adenocarcinoma. To our knowledge, the formation of these pleural cysts by

metastatic carcinoma has not yet been reported in the literature. We propose two possible explanations for cyst formation by metastatic lesions: 1) localized edema in the submesothelial space due to carcinomatous obstruction of superficial vessels in the pleura caused pleural cysts; and 2) metastatic cancer cells in the pleura produced serous fluid in the submesothelial space and formed cystic lesions. The elucidation of its etiology, however, requires the accumulation of additional cases. Thoracic oncologists and pathologists should be aware of the varied gross manifestations of metastatic adenocarcinoma to the pleura and should bear in mind the differential diagnoses of pleural cysts.

Multidisciplinary Treatment for Advanced Invasive Thymoma with Cisplatin, Doxorubicin, and Methylprednisolone

Kohei Yokoi, MD,* Haruhisa Matsuguma, MD,* Rie Nakahara, MD,* Tetsuro Kondo, MD,†
Yukari Kamiyama, MD,† Kiyoshi Mori, MD,† and Naoto Miyazawa, MD*

Background and Objectives: Advanced invasive thymomas are not usually manageable by surgical resection and radiotherapy. We reviewed our experience with a multidisciplinary approach and evaluated chemotherapy in the treatment of invasive thymoma.

Patients and Methods: Seventeen consecutive patients with invasive thymoma were treated with multimodality therapy consisting of chemotherapy, surgery, and/or radiotherapy. Four patients had stage III disease with superior vena cava invasion, nine had stage IVa disease, and four had stage IVb disease. The chemotherapy regimen consisted of cisplatin, doxorubicin, and methylprednisolone (CAMP). Chemotherapy was administered in a neoadjuvant setting to the 14 patients and in an adjuvant setting to the remaining three patients. Surgical resection was intended in all patients. After those treatments, chemotherapy and/or radiation therapy were performed.

Results: All but one of the 14 patients with induction chemotherapy responded to the CAMP therapy, and the response rate was 92.9%. Seven of these patients underwent complete remission after surgical resection and chemoradiotherapy, and the others underwent partial remission. All three patients treated with surgical resection and then chemotherapy with or without radiotherapy also achieved complete remission. Tumor progression after multimodality therapy occurred in 10 patients. After retreatment, eight of these patients were alive at the time of analysis, with a median survival time after recurrence of 30 months. The 5- and 10-year overall survival rates for all patients were both 80.7%. The major side effect of CAMP therapy was acceptable neutropenia.

Conclusions: CAMP therapy was highly effective for invasive thymomas, and the multimodality therapy containing this chemotherapy brought about good disease control in the majority of patients. We believe that this multidisciplinary treatment with CAMP therapy, surgery, and radiotherapy is a justifiable initial treatment for patients with advanced invasive thymoma. Furthermore, appropriate treatments are essential for the long-term survival of patients with recurrences after multimodality therapy.

Key Words: Thymoma, Chemotherapy, Multimodality treatment.

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In patients with thymoma, surgical resection with or without radiation therapy has been advocated as the treatment of choice for early-stage diseases.[1-3] Nevertheless, advanced-stage diseases such as tumors with great vessel invasion, pleural and/or pericardial dissemination, lymph node involvements, or distant metastases are difficult to manage by surgery and radiotherapy, and the treatment strategy for those diseases remains controversial.[4,5]

Chemotherapy has been shown to have significant antitumor activity against unresectable, recurrent, or metastatic thymomas.[6-9] Recently, multimodality therapy using chemotherapy has been examined in the treatment of advanced thymomas.[10-12] Investigators have demonstrated that combined-modality therapy can improve outcomes for advanced thymoma patients. Nevertheless, the chemotherapy regimens and treatment schedules in these studies were varied, and an optimal treatment strategy has not yet been determined. Furthermore, although it is well known that thymoma has a slow-growing nature and a late recurrent tendency, few reports contained longer follow-up data or results of retreatment of recurrences.[13-15]

To improve the outcome of patients with advanced invasive thymomas, we have conducted a study of multimodality therapy including chemotherapy. Here, we report the results with a longer follow-up.

PATIENTS AND METHODS

From February 1988 to September 2003, 38 patients with thymoma were referred to our hospital. Their clinical characteristics are shown in Table 1. Of these patients, 17 consecutive patients with advanced invasive thymoma, (four patients with stage III disease, nine with stage IVa disease, and four with stage IVb disease) including four patients with recurrent tumor, were enrolled in the study of multimodality therapy including chemotherapy, surgery, and/or radiotherapy. In all but three patients, pathologic diagnosis of thymoma was obtained by thoracotomy, transthoracic needle biopsy, or fiberoptic bronchoscopic biopsy before initiation of treatment. Among the patients without pretreatment histologic diagnosis, one patient had multiple recur-

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TABLE 1. Profile of Patients with Thymoma

Sex	
Male	17
Female	21
Age (yr)	
Median (range)	57 (25–75)
World Health Organization tumor type	
A	2
AB	6
B1	3
B2	22
B3	5
Masaoka stage	
I	15
II	4
III	6
IVa	9
IVb	4

rent pleural tumors after surgical treatment and chemotherapy for thymoma, and the remaining two had anterior mediastinal mass suspected invasive thymoma on computed tomography (CT) that were located at unsuitable places for needle biopsy. Clinical staging was determined by the medical history and physical examination, chest radiography, and chest CT. Other imaging modalities such as magnetic resonance imaging, echocardiography, or venography were performed when indicated. The staging was based on the Masaoka staging system.[16] All patients gave written informed consent for the study.

The treatment strategy of the multimodality therapy was as follows: (a) If a tumor of stage III with invasion to the great vessels or stage IV disease was distinctly demonstrated on diagnostic imaging at the initial staging, induction chemotherapy was conducted. After three or four cycles of the chemotherapy, surgical resection was attempted when the residual tumor was found, and consolidation chemotherapy and/or radiotherapy were given. (b) When stage IV disease was found on operation despite a clinically earlier stage, surgery for debulking the tumor was attempted. After that, chemotherapy was administered as a postsurgical adjuvant treatment, and then radiation therapy was applied if indicated.

The chemotherapy regimen consisted of cisplatin (20 mg/m² per day, continuous infusion on days 1 through 4), doxorubicin (40 mg/m² intravenously on day 1), and methylprednisolone (1000 mg/day intravenously on days 1 through 4 and 500 mg/day intravenously on days 5 and 6) (CAMP). Treatment cycles were repeated every 21 to 28 days. Prophylactic granulocyte colony stimulating factor was not routinely used. Surgery was intended through a median sternotomy in all patients. Resection was defined as complete (R0) if all gross disease was removed and if all surgical margins were free of the tumor. An incomplete resection meant that the surgical margins were microscopically positive (R1) or that gross residual tumors (R2) were left at the end of the operation. Radiation therapy was administered to the mediastinal

or residual tumor areas using opposite anterior and posterior parallel fields and doses of more than 50 Gy. When malignant pericardial effusion was noted during the operation, whole mediastinal irradiation was carried out.

The patients were evaluated with CT for response after induction chemotherapy and completion of the multimodality treatment. A complete remission (CR) was defined as the complete disappearance of all objective evidence of disease on CT for at least 4 weeks. A partial remission (PR) was defined as a decrease of at least 50% in the sum of the product of the perpendicular diameter of measurable lesions for at least 4 weeks. Disease progression was defined as an increase of at least 25% in tumor size or new lesions. All other circumstances were classified as no change (NC).

Survival was measured from the first day of treatment until death or the last date of the follow-up (March 31, 2004). The survival curves were calculated according to the Kaplan-Meier method, and comparisons among the curves were made by means of the log-rank test. The median follow-up time of all patients (*n* = 17) was 54 months (range, 2–193 mo), and median follow-up time of surviving patients (*n* = 14) was 62 months (range, 6–193 mo).

RESULTS

Of the 17 patients, eight were women and nine were men, ranging in age from 25 to 72 years (median, 51 yr) (Table 2). Pretreatment pathologic diagnoses were obtained in 14 patients, and the tumor histology of the remaining three patients (patients 15–17) was revealed after chemotherapy and surgical treatment. Histologic types of the thymoma were B2 tumor in 14 patients and B3 tumor in three patients, according to the World Health Organization classification.[17] All four patients who were diagnosed as having stage III disease were found to have a tumor with superior vena cava invasion on diagnostic imaging. Nine patients with stage IVa disease had pleural tumor dissemination and/or pericardial effusion, and four with stage IVb disease had pulmonary metastasis or lymph node involvement.

A summary of treatments and outcomes is listed in Table 3. CAMP therapy was administered in a neoadjuvant setting to 14 patients (Figures 1 and 2). One complete response and 13 partial responses were obtained, with an overall response rate of 92.9% (95% confidence interval [CI], 66.1–99.8%). After chemotherapy, nine patients underwent surgical resection of the residual tumor with curative intent. However, R0 resection was performed in only two patients, R1 resection in one patient, and R2 resection in six patients. Postsurgical radiotherapy was performed in eight patients. Among the remaining four patients, one complete responder for CAMP therapy had no additional treatment. Two partial responders received radiotherapy because of the unresectable tumor, and the other one refused further treatment.

Three patients (patients 1, 2, and 11) who were categorized at the initial staging as having stage I to III disease were found on operation to have stage IVa disease with pleural dissemination or malignant pericardial effusion. The patients underwent resection of the main tumor and extended

TABLE 2. Characteristics of Patients with Advanced Invasive Thymoma

Patient No.	Age (yr)	Sex	Histology	Disease Stage	Site of Disease
1	40	M	B2	IVa	Pleural dissemination
2	59	F	B2	IVa	Pericardial effusion, pericardium, aorta, lung
3	72	M	B2	IVa	Pericardial effusion, pericardium, SVC, lung
4	63	M	B2	IVb	Mediastinal lymph nodes, pleural effusion
5	38	F	B2	III	SVC
6	33	M	B2	IVa	Pleural dissemination, lung
7	65	F	B2	IVb (rec)	Pulmonary metastasis, pleural dissemination
8	66	F	B2	IVb (rec)	Pulmonary metastasis
9	62	F	B2	III	SVC
10	56	M	B3	IVa (rec)	Pleural dissemination
11	29	M	B2	IVa	Pleural dissemination, pericardium, lung
12	49	M	B3	IVa	Pleural dissemination, pericardium, pulmonary artery
13	51	F	B2	III	SVC, lung
14	62	F	B3	IVa	Pleural dissemination
15	25	M	B2	IVa (rec)	Pleural dissemination
16	29	M	B2	IVb	Pulmonary metastasis
17	62	F	B2	III	SVC

Rec, recurrent case; SVC, superior vena cava.

TABLE 3. Summary of Treatments

Patient No.	Previous Treatment	Cycles of CAMP Therapy	Response to CAMP Therapy	Subsequent Treatment	Total Response	Sites of Tumor Progression	Progression-Free Survival (mo)	Treatment for Recurrences	Overall Survival (mo)
1	S (R2)	4	NA		CR	Pleura	61	S (R0)	193+
2	S (R2)	4	NA	RT	CR		180		180+
3		4	PR	S (R1), CAMP × 2, RT	CR	Pleura, lung	45	RT	180+
4		4	PR	S (R2), RT	PR	Pericardium	11	CT[1]	13
5		4	PR	S (R0), RT	CR		169		169+
6		2+CT[2]	PR	S (R2), RT	PR	Pleura	17	CT[2]	18
7		2	PR		PR		2		2
8		3	CR		CR	Pulmonary metastasis	7	S (R0)	88+
9		2	NC	S (R2), RT	PR	Primary site	42	RT	72+
10		4	PR	RT	CR	Pleura	32	RT	67+
11	S (R2)	4	NA		CR	Pleura	24	CAMP × 2, S (R0)	56+
12		4	PR	RT	PR		54		54+
13		4	PR	S (R0)	CR		43		43+
14		4	PR	S (R2), RT	CR	Pleura	23	CAMP × 4	37+
15		4	PR		PR	Pleura	18	CAMP × 4, S (R0)	29+
16		4	PR	S (R2), RT	CR		9		9+
17		4	PR	S (R2), RT	PR		6		6+

CR, complete remission; CT[1], CDDP+VLB+BLM; CT[2], CPA+ADM+VCR+prednisone; NA, not assessable; NC, no change; PR, partial remission; R0, complete resection; R1, microscopically incomplete resection; R2, macroscopically incomplete resection; RT, radiation therapy; S, surgery.

thymectomy combined with a partial resection of the pericardium, parietal pleura, and/or lung. Even after the resection, patients 1 and 11 retained numerous miliary pleural tumors in the hemithorax, and patient 2, with malignant pericardial effusion, had a residual mass on the aortic arch. These patients received four cycles of CAMP therapy after surgery, and only patient 2 underwent subsequent whole mediastinal radiation therapy.

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FIGURE 1. Patient 5 before chemotherapy. (*A*) CT scan showing a large anterior mediastinal tumor invading the superior vena cava. (*B*) Venous phlebogram illustrating an almost complete obstruction of the superior vena cava at the level of the junction of bilateral brachiocephalic veins.
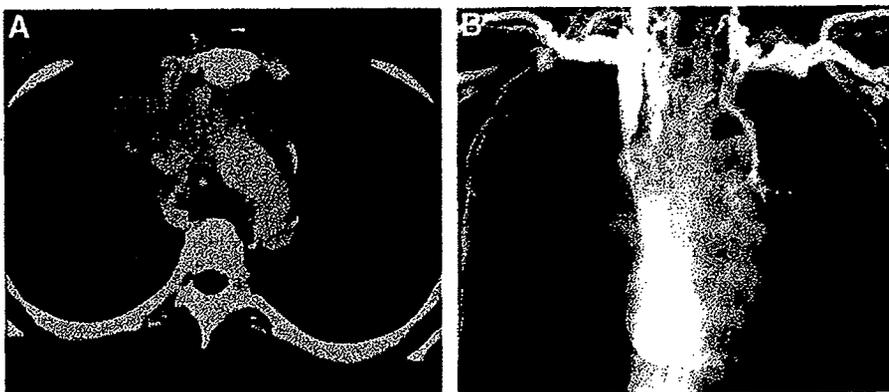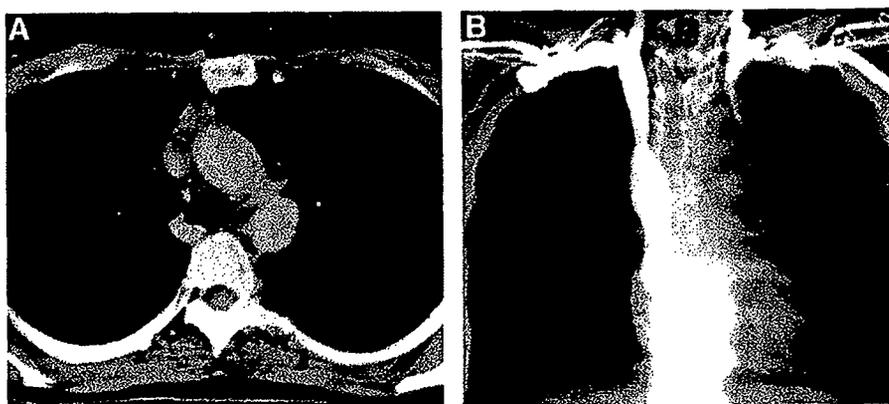

FIGURE 2. Patient 5 after four cycles of induction chemotherapy. (*A*) CT scan revealing considerable shrinkage of the tumor. (*B*) Venous phlebogram demonstrating the marked improvement of superior vena cava obstruction.

After completion of the multimodality therapy, 10 patients achieved CR and seven achieved PR; the overall remission rate was 100%. Tumor progression after treatment was observed in six (60%) of 10 CR patients and in four (57%) of seven PR patients, with a median progression-free survival of 24 months (range, 7–61 mo). The remaining six patients (four CR patients and two PR patients), 35% of the total population, had no tumor progression six to 180 months after the initiation of the multimodality therapy.

Treatment for recurrences was performed in all 10 patients. Complete surgical resection for the recurrences with or without preoperative CAMP therapy was accomplished in four patients. Patients 1 and 15 underwent an extrapleural pneumonectomy for pleural dissemination. Patient 8, who had recurrence after extrapleural pneumonectomy for the primary tumor, had a wedge lung resection for pulmonary metastasis, and patient 11 received a partial pleurectomy. For patients with unresectable recurrent tumors, radiotherapy was performed in three patients, and chemotherapy was performed in three patients whose tumors were unsuitable for radiotherapy. Two of the patients treated with chemotherapy died during the retreatment, one from recurrent tumor and the other from fulminant rhabdomyolysis.[18]

The 5- and 10-year overall survival rates of all patients were both 80.7% (95% CI, 60.9–100%) (Fig. 3). The survival curves according to stages of disease are shown in Figure 4. The 10-year survival rates of patients with stage III and stage IVa disease were 100 and 88.9% (95% CI, 68.4–100%),

respectively. In stage IVb, the 5-year survival rate was 37.5% (95% CI, 0–93.6%), and only patient 8 survived for more than 5 years after CAMP therapy and resection for recurrence. In the 10 patients with recurrence, the median survival time and 5-year survival rate after retreatment were 30 months (range, 1–132 mo) and 30.0% (95% CI, 1.6–58.4%), respectively.

Toxicity of CAMP Therapy and the Multidisciplinary Treatment

The side effects of CAMP therapy are shown in Table 4. Seventy-one cycles were administered (median, four cycles;
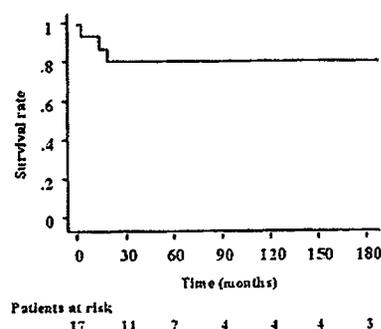

FIGURE 3. Overall survival of patients with advanced invasive thymoma who were treated with the multimodality therapy.
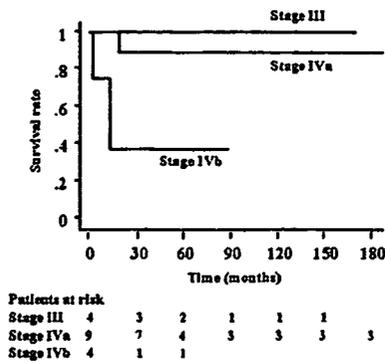
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FIGURE 4. Survival according to the Masaoka staging system. In univariate analysis, there was a significant difference between stage IVa and stage IVb disease ($p = 0.036$), but there were no significant differences between stage III and stage IVa disease ($p = 0.564$) and stage III and IVb disease ($p = 0.123$).

TABLE 4. Toxic Effects of Cisplatin, Doxorubicin, and Methylprednisolone Therapy

NCI-CTC grade (%)	0	1	2	3	4	5
Leukocytes	14	12	39	27	8	
Neutrophils	10	9	21	34	26	
Hemoglobin	75	12	11	3		
Platelets	55	36	6	3		
Nausea/vomiting	31	26	36	5	1	
Infection	92	3		3	1	1

range, two to eight cycles), and the major adverse effects were leukopenia and neutropenia. Although 60% of cycles were associated with grade 3 or 4 neutropenia, almost all patients in the study received no granulocyte colony stimulating factors or no dose reduction of all three drugs. Treatment delays (median, 1 wk; range, 1–6 wk) were performed in eight patients because of neutropenia and patients' wishes. Chemotherapy-related death occurred in patient 7. She had multiple pulmonary metastases and pleural recurrences complicated with myasthenia gravis, pure red cell aplasia, and hypogammaglobulinemia. She died of pneumonia after the second cycle. Another peculiar complication of tumor lysis syndrome developed in patient 6, with a huge thymoma of predominantly lymphocytic type during the first cycle.[18]

After CAMP therapy and surgical treatment, mild cardiac dysfunction was observed in two patients (patients 2 and 3[19]) who received whole mediastinal irradiation because of malignant pericardial effusion. No other severe complications were encountered.

DISCUSSION

Complete surgical resection is considered essential in the treatment of thymomas, even for advanced diseases and recurrences.[1–3] Nevertheless, 20 to 40% of patients who undergo surgery for thymoma receive incomplete resection or biopsy alone.[1–3] Moreover, at the initial staging, some lesions

are regarded as unresectable; these are usually advanced stage III or stage IV diseases, which are treated with chemotherapy and/or radiotherapy.[6–9]

We originated this aggressive multimodality therapy in February 1988 to improve the survival of patients with advanced or recurrent thymoma. In our study, eligible patients were limited to those with stage III lesions with great vessel invasion, stage IV lesions, or recurrences, because those tumors are not usually manageable by surgery and radiotherapy and are associated with unsatisfactory outcomes.[1–5] Our original chemotherapy regimen for invasive thymoma was designed from single-agent responsiveness for thymoma, which showed that cisplatin, doxorubicin, and corticosteroids had been the most active drugs.[20] Chemotherapy was not only administered in a neoadjuvant setting but also in a postsurgical adjuvant setting, because the initial stagings have not always been accurately estimated, even with CT and magnetic resonance imaging.

Neoadjuvant chemotherapy for invasive thymoma has been attempted in the treatment of locally advanced diseases because of the effectiveness of combination chemotherapy.[10–15] The chemotherapy regimens administered have been diverse, but almost all have included cisplatin and doxorubicin/epirubicin. The reported response rates have been documented to be 69 to 100%, and some patients receiving the treatment have had complete histologic remission. After induction chemotherapy for advanced tumors, the complete resection rates were around 70%. Of patients receiving the multimodality therapy using induction chemotherapy for locally advanced invasive thymoma, 5-year overall survival rates were reported to be between 55 and 95%,[13–15] because the study populations and treatment strategies were different.

In our 14 patients with neoadjuvant therapy, the response rate of CAMP therapy was 92.9%, which was better than or comparable with those of previous reports.[6–15] However, only two patients underwent complete resection, and seven underwent incomplete resection. The other tumors were interpreted as being unresectable after induction chemotherapy. Even after postsurgical radiotherapy, four patients without complete resection remained in PR, and two of them had a short survival. Our low complete resection rate is considered to be a result of the far advancement of the tumors: 13 of 17 patients had stage IV disease and/or recurrent tumors. Furthermore, CT was still incapable of predicting the possibility of performing a radical excision of the tumors after induction chemotherapy.

Patients undergoing incomplete resection or biopsy have been reported to show a significantly shorter survival than those with complete resection.[1–3] Blumberg et al.[2] reported that survival rates in patients with partial resection had been documented at 70 and 28% for 5 and 10 years, and 38 and 24% for biopsy, respectively. All three of our patients who had stage IV disease and were treated with surgery and then adjuvant chemotherapy with or without radiotherapy had distinct residual tumors after the operation. After the adjuvant therapy, two patients had pleural recurrences, but only after disease-free intervals of more than 5 and 2 years, respectively. In the remaining patient, postoperative CAMP therapy

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and irradiation have managed the residual disease for more than 10 years. From our available data of those patients with the adjuvant therapy, we think that aggressive postsurgical treatment including chemotherapy is useful to cure or control residual lesions in patients with incomplete resection of the primary tumors, effectively maintaining their quality of life for a longer period.

In the multimodality therapy, some complications were noted. With chemotherapy, fatal infection and tumor lysis syndrome were observed in peculiar patients with parathymic syndrome of hypogammaglobulinemia and extensive lymphocytic thymoma associated with peripheral blood T-cell lymphocytosis,[18] respectively. No mortality was encountered in surgical treatment. After radiation therapy, mild cardiac dysfunction was observed in two patients who had whole mediastinal irradiation for malignant pericardial effusion.[19] This complication is probably caused by doxorubicin and radiation affecting the heart muscle synergistically. On the whole, we think that this multimodality therapy is tolerable as long as attention is paid to any peculiar conditions.

For the recurrent tumors in six patients exhibiting CR, we aggressively performed retreatment. Extrapleural pneumonectomy or partial pleurectomy was carried out in three patients with pleural recurrences, pulmonary metastasectomy was carried out in one patient who was in a postpneumonectomy state, and repetitive radiotherapy was carried out in two patients with mediastinal or diaphragmatic local recurrences. All six patients are still in good general condition 37 to 193 months after the initial treatment. From our experience, we consider that aggressive retreatment for recurrences even after the multimodality therapy is very important for controlling disease and maintaining good quality of life, as previous reports have also advocated.[21,22]

The treatment of advanced thymoma is still controversial. However, investigators have recently advocated the necessity of multimodal approaches to therapy that introduce the enhancement of tumor resectability, cure rate, and/or long-term disease control.[10–15] In studies of such multidisciplinary treatment, Shin et al.[12] and Kim et al.[15] have reported excellent results in the survival of patients with stage III or IV thymoma. Their study protocol was considered a precise long-term treatment, which consisted of induction chemotherapy (cisplatin, doxorubicin, cyclophosphamide, and prednisone), surgical resection, postoperative radiotherapy, and consolidation chemotherapy. From our study, we also recognize the importance of postsurgical adjuvant therapy for patients with advanced disease and/or incomplete resection as well as the importance of retreatment for recurrences after the multimodality therapy. Future studies on the treatment of advanced invasive thymoma should follow a meticulous scheme of a primary multidisciplinary approach to therapy and retreatment of recurrences.

In conclusion, CAMP therapy was highly effective for invasive thymomas. Although this study was limited by its small number of patients and its nonrandomized clinical trial design, we believe that the multimodality therapy containing this chemotherapy is justifiable for the initial treatment of patients with advanced thymoma such as stage III disease

with major vessel invasion, stage IV disease, and recurrence. Further studies are warranted to determine the optimal treatment strategy.

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ORIGINAL ARTICLE

Toshio Shimizu · Taroh Satoh · Kenji Tamura
Tomohiro Ozaki · Isamu Okamoto · Masahiro Fukuoka
Kazuhiko Nakagawa

Oxaliplatin/fluorouracil/leucovorin (FOLFOX4 and modified FOLFOX6) in patients with refractory or advanced colorectal cancer: post-approval Japanese population experience

Abstract
Background. The oxaliplatin/fluorouracil/leucovorin (FOL-FOX regimen) is an effective and generally well-tolerated regimen in Western clinical studies of advanced colorectal cancer. In Japan, oxaliplatin was approved in April 2005.
Methods. To evaluate the objective tumor responses and feasibility (toxicities) of FOLFOX regimens (FOLFOX4 and modified FOLFOX6, mFOLFOX6) in a predominantly Japanese population with refractory or advanced colorectal cancer in Japan, 51 consecutive patients with histologically confirmed metastatic colon or rectum cancer who were treated between April 2005 and March 2006 were enrolled in a retrospective study. FOLFOX4 was used for treatment in 39% (first-line, 45%) of these patients, and mFOLFOX6 was used for treatment in 61% (first-line. 61%). Tumor responses were assessed radiologically, and toxicities were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 regarding toxicities other than peripheral sensory neuropathy.
Results. The objective response rates (in those who underwent first- or second-line therapy) were 50.0% and 8.7%, respectively. The tumor control rate (partial response [PR] + stable disease [SD]) was 80.4%. There were no toxicity-related deaths. Neutropenia grade 3 was experienced in 20% of patients, and often caused delay in the subsequent treatment course. Mild to moderate cumulative peripheral sensory neuropathy affected 78% of patients. The incidence of hypersensitivity reactions to oxaliplatin in our study was lower than that in reported in Western countries.
Conclusion. Both FOLFOX regimens have good efficacy in refractory or advanced colorectal cancer in a Japanese population, with an acceptable overall toxicity profile.

T. Shimizu (✉) · K. Tamura · T. Ozaki
Department of Medical Oncology, Kinki University Nara Hospital,
1248-1 Otoda-cho, Ikoma, Nara 630-0293, Japan
Tel. +81-743-77-0880; Fax +81-743-77-0890
e-mail: tshimizu@nara.med.kindai.ac.jp

T. Satoh · I. Okamoto · M. Fukuoka · K. Nakagawa
Department of Medical Oncology, Kinki University School of
Medicine, Osaka, Japan

Introduction

In 2000, it has reported that colorectal cancer (CRC) was diagnosed in more than 90000 patients per year in Japan, resulting in 36000 deaths per year.

Colorectal cancer accounts for 10% to 15% of all cancers and is the third leading cause of cancer-related death in Western countries. Approximately one-half of all patients develop metastatic disease. The prognosis for these patients is poor, although palliative chemotherapy has been shown to be able to prolong survival and improve the quality of life over best supportive care. For many years, the treatment of metastatic colorectal cancer was restricted to 5-fluorouracil (5FU) and the biomodulation of this agent.[1] Oxaliplatin and irinotecan, combined with continuous infusion of 5FU, significantly improved response rate, progression-free survival (PFS), and overall survival.[2–4] FOLFOX4 (oxaliplatin and leucovorin [LV] 5FU2) is more active than LV5FU2 alone, and has also shown superiority over IFL (irinotecan, FU bolus, leucovorin). Oxaliplatin (L-OHP), a new third-generation 1,2-DACH-platinum derivative, has a mechanism of action similar to that of other platinum derivatives.[5–9] However, its spectrum of antitumor activity in tumor models differs from those of cisplatin and carboplatin. In addition, it has also been observed to demonstrate activity against cisplatin-resistant colon carcinoma cell lines.[10] In addition, experimental data have shown synergistic activity of the oxaliplatin/FU combination. The clinical toxicity of oxaliplatin is also distinct from that of other platinum drugs: it has no renal toxicity and minimal hematotoxicity; it causes both a reversible acute, cold-related dysesthesia and a dose-limiting cumulative peripheral sensory neuropathy that usually regresses rapidly after treatment withdrawal. The recent availability of five active chemotherapeutic agents has doubled the median overall survival for metastatic CRC from 10 to 20 months.