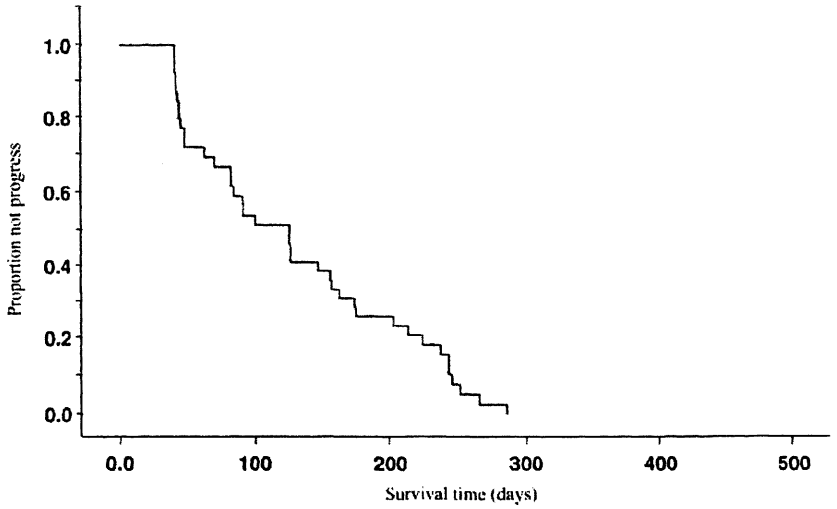

Figure 1. Kaplan–Meier survival plots for Time to Progression (TTP) in metastatic colorectal cancer (CRC) treated with certuximab plus irinotecan.

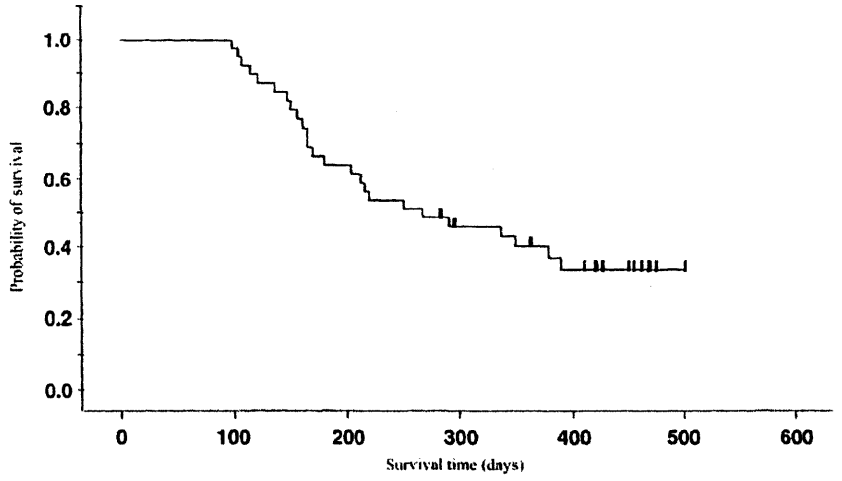

Figure 2. Kaplan–Meier survival plots for overall survival (OS) in metastatic CRC treated with certuximab plus irinotecan.

dose intensity in all patients was 45 mg/m^2 per week (range 25–61). According to the irinotecan schedule, it was 42 mg/m^2 per week (range 25–59) in those receiving it weekly and 46 mg/m^2 per week (range 29–61) in those receiving it every 2 weeks.

DISCUSSION

In this multicenter Phase II study of metastatic CRC, the combination of cetuximab plus irinotecan showed substantial efficacy as third-line treatment in patients failing previous irinotecan-, oxaliplatin- and fluoropyrimidine-based chemotherapy.

The present study was based on a Phase I study in Japan of single-agent cetuximab in subjects with solid tumors in whom standard therapy had failed (13). In that study, no DLTs and good tolerance were observed at the recommended dose, which was obtained from previous Phase II studies in Western countries. Further, the safety data for cetuximab did not obviously differ from those in Western patients and the pharmacokinetic profile was comparable. Further, another study demonstrated a lack of drug–drug interactions between cetuximab and irinotecan, and the absence of any contribution of one to the safety profiles of the other (14).

On these bases, we selected the same doses as those used overseas as the recommended dose of cetuximab in this Phase II study, namely 400 mg/m^2 initial dose and 250 mg/m^2 weekly thereafter. The incidence of Grade 3 or worse acne was 5.1%, which was lower than the 9.4–29.1% range in previous studies, whereas the incidence and severity of other non-hematological toxicities were comparable. No patients experienced Grade 3 or worse infusion reactions despite the use of antihistamines only as premedication. In a large multinational Phase II study, combination with

Table 3. Adverse events

	All ($N = 39$)	
	≥G1 (%)	≥G3 (%)
Hematological toxicities		
Leukopenia	25 (64.1)	4 (10.3)
Neutropenia	21 (53.8)	9 (23.1)
Anemia	18 (46.2)	4 (10.3)
Thrombocytopenia	4 (10.3)	0
Non-hematological toxicities		
Anorexia	30 (76.9)	6 (15.4)
Nausea	23 (59.0)	1 (2.6)
Vomiting	15 (38.5)	0
Diarrhea	30 (76.9)	7 (17.9)
Fatigue	26 (66.7)	2 (5.1)
Stomatitis	26 (66.7)	0
Hyperbilirubinaemia	9 (23.1)	3 (7.7)
AST	15 (38.5)	5 (12.8)
ALT	14 (35.9)	3 (7.7)
Hypomagnesaemia	20 (51.3)	1 (2.6)
Alopecia	19 (48.7)	0
Skin reaction		
Acne	34 (87.2)	2 (5.1)
Rash	25 (64.1)	0
Dry skin	21 (53.8)	0
Paronychia	21 (53.8)	0

AST, aspartate aminotransferase; ALT, alanine aminotransferase.

corticosteroids and antihistamines reduced the incidence of severe infusion reactions compared with the effect with antihistamines only (15). Although the incidence of Grade 3 or worse neutropenia was higher in patients receiving irinotecan every 2 weeks than in those receiving it weekly (27.6 versus 10.0%), no febrile neutropenia or life-threatening adverse events were observed in any patient. These results indicate that the combination of cetuximab and irinotecan is well-tolerated in Japanese patients with metastatic CRC refractory to irinotecan, oxaliplatin and fluoropyrimidines.

Several recent clinical trials of single-agent cetuximab in CRC refractory to irinotecan have shown similar objective RR, disease control rates, median TTP and median OS, with ranges from 8 to 12%, 32 to 50%, 1.4 to 4.2 months and 6.4 to 7.0 months, respectively (12,16–18). A more recent randomized Phase III trial of cetuximab plus best supportive care (BSC) versus BSC alone in patients with pre-treated metastatic EGFR-expressing CRC (NCIC CTG CO.17) demonstrated a significantly prolonged PFS and OS with cetuximab compared with BSC alone, indicating that cetuximab is the first targeted therapy to show a survival benefit as a single agent in metastatic CRC (17).

Moreover, the combination of cetuximab plus irinotecan has been evaluated in clinical trials in patients with previously treated metastatic CRC, including one randomized and three non-randomized trials (11,12,15,19). These studies showed a similar objective RR, disease control rate, median TTP and median OS, ranging from 17 to 25.4%, 55.5 to 63.6%, 4.1 to 4.7 months and 8.6 to 9.8 months, respectively.

Here, we showed an RR of 30.8% and a disease control rate of 64.1%. Moreover, median TTP was 4.1 months, median OS was 8.8 months and median duration of response was 5.4 months. These results indicate that combination therapy with cetuximab and irinotecan produced notable antitumor activity in metastatic CRC patients who had been pretreated with irinotecan, oxaliplatin and fluoropyrimidine, confirming the results of previous studies (11,12,15,19).

With regard to first-line treatment of metastatic CRC, a Phase II trial of cetuximab in combination with fluorouracil, leucovorin and oxaliplatin (FOLFOX-4) reported the encouraging RR of 72%, median PFS of 12.3 months and median OS of 30.0 months (20). Further, a randomized trial of FOLFIRI with or without cetuximab (the CRYSTAL trial) demonstrated a statistically significant higher overall RR (46.9 versus 38.7%, $P = 0.0038$), longer median PFS (8.9 versus 8.0 months, $P = 0.0479$) and higher surgical resection rate after chemotherapy (2.5 versus 6.0%) for the cetuximab combination arm (21). Further studies in these first- or second-line settings are required.

Among other results, we found that the RR of patients who had achieved a response in a prior irinotecan regimen was significantly higher than that of patients who had not, though the difference in survival was not significant. This result suggests that cetuximab reversed resistance to irinotecan and thereby enhanced its antitumor activity. Although this combination showed sufficient antitumor activity as third-line treatment in patients who had not achieved a prior response to irinotecan, greater clinical benefit would be obtained in those who had. Among previous studies, while the BOND study reported a subgroup analysis by progression during or within 4 weeks after pre-study with irinotecan, no study has reported a subgroup analysis by response to a prior regimen (12). The small number of patients notwithstanding, our present study is the first clinical trial of cetuximab and irinotecan for metastatic CRC to conduct a subgroup analysis by response to a prior irinotecan regimen.

Interestingly, we saw no correlation between response and the degree of EGFR expression by immunohistochemistry (IHC). Previous studies showed that EGFR expression detected by IHC was not a consistent predictor of response to EGFR-targeted therapy, and that EGFR-non-detectable patients also achieved a response to EGFR-targeted therapy (12,16). Proposed explanations for this lack of a consistent correlation include inaccuracies linked to IHC testing resulting from potential sample degradation or epitope loss during fixation. On the basis of these findings, the NCCN considers that the evaluation of EGFR expression by IHC is not of

predictive value in the determination of indications for anti-EGFR monoclonal antibody therapy (6).

With regard to adverse findings, a number of studies have demonstrated that the presence and severity of rash strongly correlate with efficacy (12,17). Here, in contrast, the presence and severity of rash were not correlated with clinical benefit. The incidence of grade 3 acne-like rash was lower than in previous studies. One reason for this lack of correlation between the presence and severity of rash and clinical benefit might be our early administration of antibiotics prior to the development of grade 3 acne-like rash. A recent randomized double-blind trial of prophylactic oral minocycline and topical tazarotene for cetuximab-associated acne-like eruption demonstrated that oral minocycline might be useful in decreasing the severity of the acneiform rash during the first month of cetuximab treatment (22). In the present study, all patients received topical agents and 51.4% received oral antibiotic drugs, including minocycline, after doctors diagnosed skin rash; median duration from the start of antibiotics to the appearance of acne-like rash was 5.7 days, which might have reduced severity.

Recent findings have confirmed an association between the expression of EGFR amplification and K-ras mutation and the efficacy to cetuximab therapy (23). Further, K-ras mutation has been shown to be an independent prognostic factor in CRC patients treated with cetuximab (24,25). While the expression of these variables was not analyzed in the present study, these findings will likely assist in future efforts to define the subpopulation of patients most likely to benefit from cetuximab.

In conclusion, this study provides evidence of the substantial clinical efficacy of combination therapy with cetuximab plus irinotecan as third-line treatment in metastatic CRC patients who are refractory to irinotecan-based chemotherapy and who have failed oxaliplatin- and fluoropyrimidine-based chemotherapies in their previous treatment.

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Conflict of interest statement

None declared.

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Appendix

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Original article

Combination chemotherapy with cisplatin and irinotecan in patients with adenocarcinoma of the small intestine

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Abstract

Background. **Small-bowel adenocarcinoma (SBA) is a rare tumor that has a poor response to chemotherapy and a poor prognosis. Treatment strategies for SBA have not been clearly established.**

Methods. **All patients with SBA treated using a combination of cisplatin and irinotecan (IP) as first-line chemotherapy at the National Cancer Center Hospital in Japan between January 1999 and February 2007 were studied retrospectively.**

Results. **Eight patients received IP as first-line chemotherapy. The median follow-up was 9.5 months (range, 4.2–37.5 months). The median number of cycles of IP was three (range, 1–5). The overall response rate (complete or partial response) was 12.5% (complete response, $n = 0$; partial response, $n = 1$). The disease control rate (complete or partial response or stable disease) was 75%. The median time to treatment failure was 4.5 months (95% confidence interval, 0.9–5.8 months), and overall survival was 17.3 months (range, 1.9–21.3 months). The most common adverse events were neutropenia and anorexia.**

Conclusion. **IP combination chemotherapy may be an acceptable option for patients with SBA. Further studies are warranted to determine the optimal chemotherapeutic regimen for SBA.**

Key words Adenocarcinoma · Small intestine · Chemotherapy · Cisplatin · Irinotecan

Introduction

Although the small bowel comprises more than 90% of the intestinal surface, small bowel malignancies are rare [1]. Small-bowel adenocarcinoma (SBA) accounts for fewer than 1% of gastrointestinal cancers [2], with an incidence of 3.9 cases per million persons, and a mean age at diagnosis of 60–70 years [3]. Given the rarity of the disease, its characterization remains limited. Crohn's disease, Peutz-Jeghers syndrome, familial adenomatous polyposis, and type 1 neurofibromatosis are known risk factors for SBA [4]. The low incidence, obscure symptoms, and difficulty in diagnosis mean that the disease often manifests in an advanced stage. Generally, advanced metastatic SBA is known to have a poor response to chemotherapy and a poor prognosis, and no standard therapeutic strategy has yet been established.

Previous reports have studied several chemotherapy regimens, including 5-fluorouracil (5-FU), platinum compounds, and irinotecan [5–9]. In one of the few prospective, phase II studies of patients with SBA, Gibson et al. [5] found that the FAM regimen (5-FU, doxorubicin, and mitomycin C) was active and tolerable for patients with advanced SBA. In that study, the response rate was 18.4%, and the median survival was 8 months. Despite the small number of retrospective studies, it appears that irinotecan may have some efficacy against SBA [6, 7].

Combination chemotherapy with irinotecan and cisplatin (IP) has been investigated as first- or second-line therapy in patients with gastric cancer. Chemotherapeutic regimens for gastric or colorectal cancer are also often effective for SBA. Therefore, we have treated patients presenting with advanced or recurrent SBA using IP as first-line chemotherapy at the National Cancer Center Hospital (NCCH). The purpose of the present study was to determine the efficacy of IP in patients with SBA and to discuss the feasibility of this approach.

Patients and methods

Patient selection

Patients were retrospectively selected for this study according to the following criteria: (1) histologically

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confirmed adenocarcinoma arising from the small intestine, except for the ampulla of Vater; (2) metastatic or recurrent disease; (3) combination therapy with IP as first-line chemotherapy; and (4) treatment performed at the NCCH between January 1999 and February 2007.

Treatment schedule

On day 1, irinotecan 70 mg/m^2 was given as a 90-min intravenous infusion, followed 2 h later, by cisplatin 80 mg/m^2 given over 120 min. Irinotecan 70 mg/m^2 was also given on day 15. Each cycle lasted 4 weeks. To avoid cisplatin-induced renal damage, patients were hydrated intravenously on day 1 with 3000 ml of 5% dextrose in 0.09% sodium chloride, and diuresis was induced with furosemide. Hydration with 2000 ml of 5% dextrose in 0.09% sodium chloride was continued for another 48 h [10].

Response and toxicity evaluation

The best response during initial chemotherapy was evaluated. Complete response (CR) was defined as the complete disappearance of all clinically detectable tumors and no new lesions. Partial response (PR) was defined as a decrease of 30% or more in the sum of the longest diameters of the target lesion (compared to the baseline sum of the longest diameters), in addition to nonprogression in nontarget lesions and no new lesions. Stable disease (SD) consisted of regression of target lesions insufficient to meet the criteria for PR, an increase of less than 20% in the sum of the longest diameters of the target lesion (compared to the smallest sum of the longest diameters after chemotherapy), no progression in nontarget lesions, and no new lesions. In patients in whom the response to the first cycle of chemotherapy was progressive disease (PD), the best response was PD. Even when the chemotherapeutic regimen was changed because of toxicity without disease progression, the best response was defined as that which occurred during first-line chemotherapy with IP.

The National Cancer Institute common toxicity criteria (version 3.0) were used for evaluation of toxicity.

Data collection and statistical analyses

Patient characteristics, including age, sex, performance status (PS), primary site and degree of differentiation of adenocarcinoma, date of initial diagnosis for metastatic or recurrent SBA, start of IP, progressive disease and death or last follow-up, as well as details of treatment obtained from medical records, were collected retrospectively. Time to treatment failure (TTF) was defined as the interval between first administration of the drugs and termination of drugs for any reason or death or last follow-up. Overall survival (OS) was defined as the interval between first administration of the drugs and death or last follow-up. Median TTF and OS were estimated using the Kaplan-Meier method.

Results

IP was given as first-line chemotherapy in eight patients at the NCCH between January 1999 and February 2007. The median follow-up was 9.5 months (range, 4.2–37.5 months). The patients' characteristics are shown in Table 1. A tendency toward female predominance was seen. The patients' median age was 61 years (range, 52–71 years), and PS was well maintained. Two patients had recurrent SBA after curative surgery and received no adjuvant chemotherapy. No patients had risk factors for SBA, such as Crohn's disease. A total of 23 cycles were given, with a median number of 3 cycles per patient (range, 1–5).

Chemotherapy and response

The overall response rate for first-line IP chemotherapy was 12.5% (PR, $n = 1$; CR, $n = 0$). Five patients had SD;

Table 1. Patient characteristics

	Patients ($n = 8$)	%
Sex		
Male	3	37.5
Female	5	62.5
Age (years)		
Median (range)	61 (56–71)	
PS		
0	6	75.0
1	2	25.0
Primary site		
Duodenum	6	75.0
Jejunum	1	12.5
Ileum	1	12.5
Differentiation		
Intestinal type	2	25.0
Diffuse type	1	12.5
Not examined	5	62.5
Disease stage		
Advanced	6	75.0
Recurrent after surgery	2	25.0
Site of metastases		
Regional lymph nodes	5	62.5
Distant lymph nodes	1	12.5
Liver	4	50.0
Lung	1	12.5
Peritoneum	1	12.5
Number of metastases		
1	5	62.5
2	3	37.5

Table 2. Detailed patient information

Patient	Age (years)	Sex	PS	Primary site	Site of metastases	Response to IP	TTF (months)	Second-line chemotherapy	OS (months)
1	71	F	0	Ileum	Regional LN	SD	2.6	Not applicable[a]	18.2
2	61	M	1	Duodenum	Regional LN	SD	4.5	Mitomycin/rinotecan	8.1
3	56	F	1	Duodenum	Liver, regional LN	PR	5.8	S-1	21.3
4	58	M	0	Jejunum	Liver, regional LN	SD	4.2	Mitomycin/irinotecan	17.3
5	56	F	0	Duodenum	Liver	PD	2.1	5-FU	4.4
6	62	F	0	Duodenum	Lung	SD	4.5	S-1	16.7[b]
7	64	F	0	Duodenum	Peritoneum, liver	PD	2.3	None	3.4
8	62	M	0	Duodenum	Regional LN	SD	0.9	S-1/cisplatin	1.9[b]

PS, performance status; IP, combination therapy with irinotecan and cisplatin; TTF, time to treatment failure; OS, overall survival; LN, lymph nodes; SD, stable disease; PR, partial response; PD, progressive disease
[a] Patient referred to another hospital
[b] Censored cases

thus, the disease control rate was 75% (six of eight patients). The patient who achieved PR was a 56-year-old woman with advanced duodenal adenocarcinoma who had positive regional lymph nodes and liver metastases (Table 2). Five courses of IP, which caused mild toxicities, were given before disease progression. Although the degree of differentiation of adenocarcinoma was examined in only three of the eight patients, whether the type was intestinal or diffuse was not likely to be associated with chemotherapy response.

The detailed characteristics of each patient are shown in Table 2. Second-line chemotherapy was received by six of seven patients (85.7%); information on second-line chemotherapy was unavailable for one patient with SD, because that patient was referred to another hospital. The remaining patient did not undergo second-line chemotherapy due to poor PS resulting from disease progression. Four of the six patients who received second-line chemotherapy achieved SD, and the other two patients had PD. Thus, no patients achieved CR or PR with second-line chemotherapy. Two of three patients who had S-1 or its combination as second-line chemotherapy seemed to have longer survival than the others and the remaining patient's result was censored and thus unknown.

TTF and OS

The median TTF was 4.5 months (95% confidence interval [CI], 0.9–5.8 months), and OS was 17.3 months (range, 1.9–21.3 months). The Kaplan-Meier curve for OS is shown in Fig. 1. Six of the eight patients died from progression of SBA. Patients with PR or SD tended to survive longer than patients with PD (Table 2).

Toxicity of IP

All eight patients were assessable for toxicity. The toxicity profiles are listed in Table 3. In terms of hematological toxicity, neutropenia was common, with severe toxicity in 25% of patients. Febrile neutropenia occurred in one patient (12.5%). Grade 3 anemia was observed in two patients and might have been associated with gastrointestinal bleeding from the primary lesion. Common nonhematological toxicities were gastrointestinal, such as anorexia, diarrhea, and nausea. Seven of the eight patients (87.5%) experienced anorexia, and two developed severe anorexia. Only one patient developed a mildly elevated serum creatinine level, but liver dysfunction was observed in two patients, with elevated serum aspartate aminotransferase, alanine aminotransferase, and bilirubin levels. In one such patient, grade 3 toxicity occurred and resulted in postponement of treatment. In all, three patients discontinued IP due to adverse effects without disease progression. Toxicities responsible for discontinuation were hearing disturbance, taste alteration, and severe anorexia. No treatment-related deaths were observed with IP.

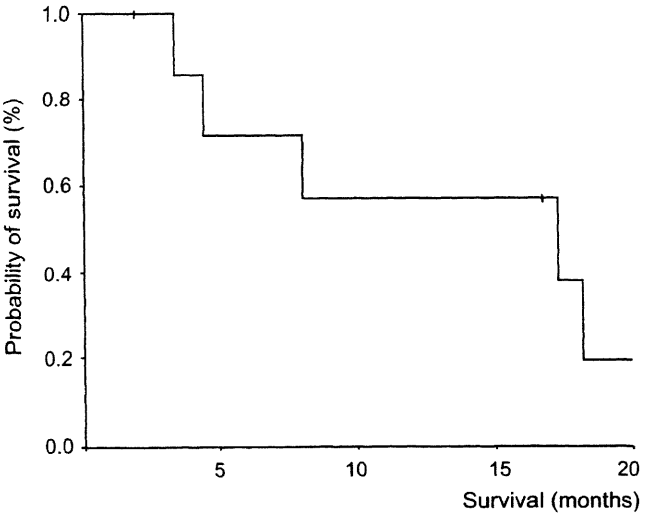

Fig. 1. Overall survival

Table 3. Toxicity in patients treated with irinotecan and cisplatin

	Grade (NCI CTC-v.3)				
	1 (*n*)	2 (*n*)	3 (*n*)	4 (*n*)	Grade 3/4 (%)
Leukopenia	0	0	1	0	12.5
Neutropenia	1	3	1	1	25.0
Anemia	0	2	2	0	25.0
Anorexia	2	3	2	0	25.0
Alopecia	1	0	0	0	0.0
Diarrhea	1	0	0	0	0.0
Fatigue	2	0	0	0	0.0
Nausea	2	2	0	0	0.0
Vomiting	1	0	0	0	0.0
Erythema	2	0	0	0	0.0
Neuropathy-hearing	0	0	1	0	12.5
Taste alteration	0	1	0	0	0.0
Febrile neutropenia	0	0	1	0	12.5
Bilirubin	0	1	0	0	0.0
AST	0	0	1	0	12.5
ALT	0	0	1	0	12.5
Creatinine	1	0	0	0	0.0

NCI CTC, National Cancer Institute common toxicity criteria, version 3; AST, aspartate aminotransferase; ALT, alanine aminotransferase

Discussion

As SBA is such a rare disease, few patients with advanced or recurrent SBA are treated each year at our institution. Because little evidence has been provided with respect to treatment of SBA, chemotherapy regimens for SBA are selected based on previous reports dealing with SBA or treatment for other gastrointestinal cancers. We have previously used combined 5-FU and cisplatin or continuous 5-FU infusion as first-line chemotherapy in patients with SBA. However, because response to these regimens was not particularly good, we changed from 5-FU-based chemotherapy to IP, which had been investigated for patients with gastric cancer as first- or second-line chemotherapy [10–12]. To date, no reports have described SBA patients treated with IP as first-line chemotherapy.

Polyzos et al. [7] reported that two of three patients with advanced SBA achieved PR and palliation of symptoms with single-agent irinotecan, while Locher et al. [6] found that second-line chemotherapy with 5-FU and irinotecan resulted in disease stabilization in 50% of patients. Fishman et al. [13] retrospectively reported that a chemotherapy regimen including irinotecan appeared to offer higher overall response rates than fluorouracil-based regimens for first- or second-line chemotherapy.

Little evidence is available concerning the role of platinum compounds (including cisplatin) in patients with SBA. Erlichman et al. [14] reported that, in a phase I study, combination treatment with irinotecan, 5-FU, leucovorin, and oxaliplatin resulted in one partial response and two patients with SD. Also, Locher et al. [6] found that combining 5-FU and platinum compounds for first-line chemotherapy resulted in an objective response rate of 21%, a median progression-free survival of 8 months, and median overall survival (OS) of 14 months, with manageable toxicities.

Although the present results were inferior to other regimens with respect to the overall response rate and progression-free survival for first-line chemotherapy, the OS was longer. In the present study, many patients received second-line chemotherapy involving fluoropyrimidine drugs. Actually, patients who had S-1 as second-line chemotherapy tended to have longer survival. This implies that, as with other gastrointestinal cancers, treating advanced SBA with all possible effective drugs, such as fluoropyrimidine drugs, irinotecan, and platinum compounds, throughout the treatment period may be important. Simultaneously, possible effective combinations of drugs should be investigated, such as S-1 based regimens.

Patients with gastric cancer show a variety of hematological and nonhematological toxicities with IP regimens [11, 12]. In a phase II study in which patients with metastatic gastric cancer received a median of three IP treatment courses (range, 1–7), grade 4 neutropenia was observed in 37%–57% of patients, and grade 3 or 4 nausea was observed in 18% [11]. In the present study, some mild and severe toxicities were also observed in patients with SBA. Thus, adequate management of toxicities and discontinuation of chemotherapy, as necessary, is important for IP regimens.

In conclusion, patients with advanced and recurrent SBA treated using combined IP for first-line chemotherapy were retrospectively studied. Combination che-

motherapy with IP may offer an acceptable option in patients with SBA, but such patients require supportive care. Further studies are warranted to identify more effective and safer antitumor drugs for SBA patients, including molecular targeted therapy.

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Short Communication

Close Association of *UGT1A9* IVS1+399C>T with *UGT1A1*28*, **6*, or **60* Haplotype and Its Apparent Influence on 7-Ethyl-10-hydroxycamptothecin (SN-38) Glucuronidation in Japanese

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ABSTRACT:

The anticancer prodrug, irinotecan, is converted to its active form 7-ethyl-10-hydroxycamptothecin (SN-38) by carboxylesterases, and SN-38 is inactivated by UDP-glucuronosyltransferase (UGT)1A1-mediated glucuronidation. UGT1A9 also mediates this reaction. In a recent study, it was reported that the *UGT1A9* IVS1+399 (I399)C>T polymorphism is associated with increased SN-38 glucuronidation both in vitro and in vivo. However, its role in UGT1A9 expression levels and activity is controversial. Thus, we evaluated the role of I399C>T in SN-38 glucuronidation using 177 Japanese cancer patients administered irinotecan. I399C>T was detected at a 0.636 allele frequency. This polymorphism was in strong linkage disequilibrium (LD) with *UGT1A9*1b* (−126_−118T_9>T_{10}, |D′| = 0.99) and *UGT1A1*6* (211G>A, 0.86), in moderate LD with *UGT1A1*60* (−3279T>G, 0.55), but weakly associated with *UGT1A1*28* (−54_−39A$(TA)_6$TAA>A$(TA)_7$TAA, 0.25). Haplotype analysis showed that 98% of the I399C alleles were linked with low-activity haplotypes, either *UGT1A1*6*, **28*, or **60*. On the other hand, 85% of the T alleles were linked with the *UGT1A1* wild-type haplotype **1*. Although I399T-dependent increases in SN-38 glucuronide/SN-38 area under concentration-time curve (AUC) ratio (an in vivo marker for UGT1A activity) and decreases in SN-38 AUC/dose were apparent ($P < 0.0001$), these effects were no longer observed after stratified patients by *UGT1A1*6*, **28*, or **60* haplotype. Thus, at least in Japanese populations, influence of I399C>T on SN-38 glucuronidation is attributable to its close association with either *UGT1A1*6*, **28*, or **60*.

Irinotecan is an important drug for treatment of various tumors including lung, colon, and gastric (Smith et al., 2006). The infused drug is metabolized to its active form 7-ethyl-10-hydroxycamptothecin (SN-38) by carboxylesterases, and SN-38 is inactivated by glucuronidation. At least four UDP-glucuronosyltransferase (UGT) isoforms, namely UGT1A1, UGT1A7, UGT1A9, and UGT1A10, are known to glucuronidate SN-38 (Gagné et al., 2002; Saito et al., 2007).

The UGT1A gene complex consists of 9 active first exons including *UGT1A10*, *1A9*, *1A7*, and *1A1* (in this order) and common exons 2 to 5. One of the 9 first exons can be used in conjunction with the common exons (Tukey and Strassburg, 2000). The UGT1A N-terminal domains (encoded by the first exons) determine substrate-binding specificity, and the C-terminal domain (encoded by exons 2 to 5) is important for binding to UDP-glucuronic acid. The 5′- or 3′-flanking region of each exon 1 is presumably involved in regulation of its expression. Substantial interindividual differences have been detected in mRNA and protein levels and enzymatic activity of the UGT1A isoforms (Fisher et al., 2000; Saito et al., 2007).

SN-38 glucuronidation is thought to be mediated mainly by UGT1A1, and its genetic polymorphisms affecting irinotecan pharmacokinetics and adverse reactions have been already identified. The TA-repeat polymorphism, −54_−39A$(TA)_6$TAA>A$(TA)_7$TAA (*UGT1A1*28* allele), is associated with lower promoter activity, resulting in reduced SN-38 glucuronidation (Beutler et al., 1998; Iyer et al., 1999). The single nucleotide polymorphism (SNP) 211G>A (Gly71Arg, **6* allele), found mainly in East Asians, causes reduced protein expression levels and SN-38 glucuronidation activity (Gagné et al., 2002; Jinno et al., 2003). Another SNP in the enhancer region of *UGT1A1*, −3279T>G (**60* allele), is also a causative factor for reduced expression (Sugatani et al., 2002). Allele frequencies have been reported for **28* (0.09–0.13), **6* (0.15–0.19), and **60* (0.26–0.32) in Japanese and Chinese populations and for **28* (0.30–0.39), **6* (~0), and **60* (0.44–0.55) in whites (Saito et al., 2007). In a previous study, in the Japanese population, we defined haplotype **28* as the haplotype harboring the **28* allele, haplotype **6* as that harboring the**6* allele, and haplotype **60* as that harboring the **60* allele (and without the **28* or **6* allele) (Sai et al., 2004; Saeki et al., 2006). Note that most of the **28* haplotypes concurrently harbored the **60* alleles, and that the **28* and **6* alleles were exclusively present on the different chromosomes (Sai et al., 2004; Saeki et al., 2006). We have also revealed that the haplotype **28*, **6*, or **60* was associated with reduced SN-38 glucuronide (SN-38G)/SN-38 area under concentration-time curve (AUC) ratios, an in vivo parameter for UGT1A activity (Minami et al., 2007).

In a recent study, an intronic SNP of *UGT1A9*, IVS1+399 (I399)C>T, has been shown to be associated with increased UGT1A9 protein levels and glucuronidation activities toward SN-38 and the UGT1A9 probe drug propofol (Girard et al., 2006). Elevation of

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ABBREVIATIONS: SN-38, 7-ethyl-10-hydroxycamptothecin; UGT, UDP-glucuronosyltransferase; SNP, single nucleotide polymorphism; SN-38G, SN-38 glucuronide; AUC, area under concentration-time curve; I399, *UGT1A9* IVS1+399; LD, linkage disequilibrium.

SN-38 glucuronidation activity by this SNP is significant among subjects without *UGT1A1*28*. Sandanaraj et al. (2008) have also reported that I399C/C patients showed higher SN-38 AUC than C/T and T/T patients. With the same *UGT1A1* diplotypes, patients with I399T/T (and *UGT1A9* −126_−118T_{10}/T_{10}) have shown higher SN-38G C_{max} than I399C/T (and T_9/T_{10}) patients. *UGT1A9*1b* (*UGT1A9* −126_−118T_9>T_{10}) has been shown to have no affect on UGT1A9 expression levels (Girard et al., 2006; Ramírez et al., 2007; Sandanaraj et al., 2008). Thus, two groups did suggest that I399T allele was associated with higher glucuronidation activity. However, using human liver microsomes, Ramírez et al. (2007) showed that I399C>T had no significant effect on both UGT1A9 mRNA levels and glucuronidation activities for two UGT1A9 substrates. Therefore, the roles of I399C>T in UGT1A9 activities as well as SN-38 glucuronidation remain inconclusive.

In the present report, we reveal the linkage of I399C>T with *UGT1A1*, *UGT1A7*, and *UGT1A9* polymorphisms and analyze its association with the SN-38G/SN-38 AUC ratio and SN-38 AUC/dose (per dose) to clarify its role in SN-38 glucuronidation.

Materials and Methods

Patients. One hundred and seventy-seven patients (81 lung, 63 colon, 19 stomach, and 14 other cancer patients) administered irinotecan at the National Cancer Center were enrolled in this study as described previously (Minami et al., 2007). This study was approved by the ethics committees of the National Cancer Center and the National Institute of Health Sciences, and written informed consent was obtained from all participants. Eligibility criteria, patient profiles, and irinotecan regimens are summarized in our previous report (Minami et al., 2007). In brief, patients consisted of 135 males and 42 females with a mean age of 60.5 (26–78 years old), and their performance status was 0 (84 patients), 1 (89 patients), or 2 (4 patients). Irinotecan administrations were conducted according to the standard protocols in Japan as follows: i.v. 90-min infusion at a dose of 100 mg/m^2 weekly or 150 mg/m^2 biweekly in irinotecan monotherapy; and 60 mg/m^2 weekly with cisplatin in most combination therapies.

Genotyping and Haplotype Analysis. Genomic DNA was extracted from whole blood of 177 irinotecan-administered patients (Saeki et al., 2006). *UGT1A9* IVS1+399C>T (rs2741049) was genotyped using the TaqMan SNP Genotyping Assay kit (C_9096281_10) according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). The *UGT1A1*28* allele [−54_−39A(TA)$_6$TAA>A(TA)$_7$TAA], *UGT1A1*6* allele [211G>A (Gly71Arg)], *UGT1A1*60* allele (−3279T>G), *UGT1A7*2* haplotype [387T>G, 391C>A and 392G>A (Asn129Lys and Arg131Lys)], *UGT1A7*3* haplotype [387T>G, 391C>A, 392G>A, and 622T>C (Asn129Lys, Arg131Lys, and Trp208Arg)], and *UGT1A9*1b* allele (−126_−118T_9>T_{10}) were determined previously (Saeki et al., 2006). Hardy-Weinberg equilibrium analysis of I399C>T, linkage disequilibrium (LD) analysis of the *UGT1A9*, *UGT1A7*, and *UGT1A1* polymorphisms, and haplotype estimation with an expectation-maximization algorithm were performed using SNPAlyze version 7.0 software (Dynacom, Chiba, Japan).

Pharmacokinetics. Pharmacokinetic data for the 176 irinotecan-treated patients (data for one patient was unavailable) were described previously (Minami et al., 2007). In brief, heparinized blood was collected before irinotecan administration and at 0, 0.33, 1, 2, 4, 8, and 24 h after termination of the first infusion of irinotecan. SN-38 and SN-38G plasma concentrations were determined by high-performance liquid chromatography, and AUC was calculated using the trapezoidal method in WinNonlin version 4.01 (Pharsight, Mountain View, CA).

Statistical Analysis. Gene dose effects of I399C>T and *UGT1A1* haplotypes (**28*, **6*, *or *60*) were assessed by the Jonckheere-Terpstra test using StatExact version 6.0 (Cytel Inc., Cambridge, MA). Multiplicity adjustment was conducted with the false discovery rate. The significant difference was set at $p = 0.05$ (two-tailed).

Results

Linkages of *UGT1A9* IVS1+399 (I399)C>T with Other Polymorphisms. In our patients, I399C>T was detected at a 0.636 allele frequency, which is almost the same as those in the HapMap data (rs2741049) for Japanese (0.663) and Han Chinese (0.633) populations, but higher than those for Europeans (0.383) and Sub-Saharan Africans (Yoruba) (0.417). Genotype distribution for this SNP was in Hardy-Weinberg equilibrium (p = 0.418). LD analysis was performed between I399C>T and the previously determined genotypes, *UGT1A9*1b*, *UGT1A7*2* and **3*, and *UGT1A1*28*, **6*, and **60*, which were detected at >0.1 frequencies in Japanese populations (Saeki et al., 2006). When assessed by the |D′| value, I399C>T was in complete LD with *UGT1A7* 387T>G, 391C>A and 392G>A (*UGT1A7*2*, |D′| = 1.000); in strong LD with *UGT1A9* −126_−118T_9>T_{10} (*UGT1A9*1b*, 0.987), *UGT1A7* 622T>C (*UGT1A7*3*, 0.977), and *UGT1A1* 211G>A (*UGT1A1*6*, 0.864); and in moderate LD with *UGT1A1* −3279T>G (*UGT1A1*60*, 0.554), but weakly associated with *UGT1A1* −54_−39A(TA)$_6$TAA>A(TA)$_7$TAA (*UGT1A1*28*, 0.252). In r^2 values, the I399C>T was in strong LD with *UGT1A7*2* (r^2 = 0.976) and *UGT1A9*1b* (0.916), in moderate LD with *UGT1A7*3* (0.478), but in weak LD with *UGT1A1*6* (0.261) and *UGT1A1*60* (0.208), and in little LD with *UGT1A1*28* (0.018).

Haplotype Analysis. Haplotype analysis was performed using the 9 polymorphisms including I399C>T. As shown in Fig. 1, 95% (123/129) of the I399C alleles were linked with the *UGT1A9* −126_−118T_9 alleles, and 100% (225/225) of the T alleles were linked with the T_{10} alleles (*UGT1A9*1b*). The I399C alleles were completely (129/129) linked with the *UGT1A7* 387G, 391A, and 392A alleles, and most T alleles (223/225) were linked with the 387T, 391C, and 392G alleles. The 40% (51/129) and 60% (78/129) of the I399C alleles were linked with *UGT1A7*2* and *UGT1A7*3* haplotypes, respectively. We also found that 98% (126/129) of the I399C alleles were linked with the *UGT1A1*6* (211G>A), **28* [−54_−39A(TA)$_6$TAA>A(TA)$_7$TAA], or **60* (−3279T>G). According to the *UGT1A1* haplotype definition by Sai et al. (2004), 42% (54/129), 36% (46/129), 19% (25/129), and 1% (1/129) of the I399C alleles were linked with the *UGT1A1* haplotypes **6a* (harboring **6* allele), **60a* (harboring **60* allele), **28b* (harboring **60* and **28* alleles), and **28d* (harboring **28* allele), respectively. On the other hand, 85% (191/225) of the T alleles were linked with the *UGT1A1* wild-type haplotype **1*.

Association Analysis. The associations of I399C>T with irinotecan pharmacokinetic parameters were then analyzed using the estimated haplotypes. First, association with SN-38G/SN-38 AUC ratio, an in vivo parameter of UGT1A activity (Sai et al., 2004; Minami et al., 2007; Sandanaraj et al., 2008), was analyzed. *UGT1A7*2* had unchanged activity for SN-38 glucuronidation (Gagné et al., 2002), and neither *UGT1A9*1b* nor *UGT1A7*3* had significant effects on the SN-38G/SN-38 AUC ratio in our previous study (Minami et al., 2007). On the other hand, the *UGT1A1*6*, **28*, and **60* haplotypes were associated with the reduced SN-38G/SN-38 AUC ratios (Minami et al., 2007). Although effects of the haplotype **28* and **6* were more striking, haplotype *UGT1A1*60*, harboring only the **60* allele without the **28* allele, was weakly associated with the reduced ratio. To remove even this weak effect and clarify the real effect of I399C>T, *UGT1A1*60* was also considered as low-activity haplotype in this analysis. Namely, we analyzed the associations of I399C>T with the AUC ratio within the groups stratified by the *UGT1A1* haplotypes, *UGT1A1*28* (**28b* and **28d*), **6* (**6a*), and **60* (**60a*) (combined and shown as *UGT1A1*"+").

When stratified by the I399C>T genotype, a T allele-dependent

Gene		*UGT1A9*		*UGT1A7*[2]				*UGT1A1*[3]			Number	Frequency
Nucleotide change		-126_-118 T_9>T_{10}	IVS1+399 C>T	387 T>G	391 C>A	392 G>A	622 T>C	-3279 T>G	$(TA)_6$>$(TA)_7$	211 G>A		
Allele name		*1b		*2, *3	*2, *3	*2, *3	*3	*60, *28	*28	*6		
Haplotypes[1]	*1C-*3-*6a										47	0.133
	*1C-*2-*60a										44	0.124
	*1C-*3-*28b										21	0.059
	*1C-*2-*28b										4	0.011
	*1C-*3-*60a										2	0.006
	*1C-*3-*28d										1	0.003
	*1C-*2-*6a										1	0.003
	*1bC-*3-*6a										6	0.017
	*1C-*2-*1										2	0.006
	*1C-*3-*1										1	0.003
	*1bT-*1-*1										190	0.537
	*1bT-*3-*1										1	0.003
	*1bT-*1-*28b										22	0.062
	*1bT-*1-*60a										5	0.014
	*1bT-*1-*6a										5	0.014
	*1bT-*1-*28d										1	0.003
	*1bT-*2-*60a										1	0.003
Allele frequency		0.653	0.636	0.370	0.370	0.370	0.223	0.280	0.138	0.167	354	1.000

FIG. 1. Haplotypes assigned by using common *UGT1A9*, *UGT1A7*, and *UGT1A1* polymorphisms. [1]Haplotypes were shown as *UGT1A9* haplotypes − *UGT1A7* haplotypes − *UGT1A1* haplotypes. Major allele, white blocks; minor allele, gray blocks. **1C*, T_9 and I399C; **1bC*, T_{10} and I399C; **1bT*, T_{10} and I399T in *UGT1A9*. [2]*UGT1A7*2* and **3* are the haplotypes harboring the three and four *UGT1A7* alleles, respectively. [3]*UGT1A1* $(TA)_6$>$(TA)_7$ indicates −54_−39A$(TA)_6$TAA>A$(TA)_7$TAA.

increase in the SN-38G/SN-38 AUC ratio was observed ($p < 0.0001$, Jonckheere-Terpstra test) (Fig. 2A). However, this trend was obviously dependent on biased distributions of *UGT1A1* haplotypes; e.g., 96% of the I399C/C patients were homozygotes for *UGT1A1*28*, **6*, *or *60*; and "*UGT1A1*28*, **6*, or **60*"-dependent reduction of SN-38G/SN-38 AUC ratio was found within the I399T/T genotypes ($p < 0.05$). As shown in Fig. 2B, *UGT1A1*28*, **6*, or **60* (*UGT1A1*+)-dependent reduction in the SN-38G/SN-38 ratio was observed when patients were stratified by these three haplotypes. However, no significant effect of I399C>T was found within the stratified patients ($p > 0.05$ within the −/−, −/+, or +/+ patient group in Fig. 2B). As for SN-38 AUC/dose (SN-38 AUC values adjusted by the doses used), a similar *UGT1A1* haplotype dependence was observed. Although the I399T-dependent reduction of SN-38 AUC/dose was detected ($p < 0.0001$), biased distributions of the *UGT1A1*28*, **6*, or **60* were again evident, and the *UGT1A1* + haplotypes-dependent increase was significant within the I399 C/T and T/T patients ($p < 0.01$ and $p < 0.05$, respectively) (Fig. 2C). Moreover, no significant effect of I399C>T on SN-38 AUC/dose was found when stratified by the *UGT1A1* haplotypes ($p > 0.05$ within the −/−, −/+, or +/+ patient group in Fig. 2D).

Discussion

In the present study, LD between I399C>T and *UGT1A1*, *UGT1A7*, or *UGT1A9* polymorphisms in Japanese populations was shown for the first time. Moreover, the apparent effect of I399C>T on SN-38 glucuronidation in Japanese cancer patients was suggested to result from its close association with *UGT1A1*28*, **6*, or **60*.

As for the influence of I399C>T on UGT1A9 activity, conflicting results have been reported. Girard et al. (2006) have shown that I399C>T was associated with increased UGT1A9 protein levels and enzyme activity toward an UGT1A9 probe drug propofol using 48 human liver microsomes derived mainly from whites. In contrast, using human liver microsomes from 46 white subjects, Ramírez et al. (2007) have revealed that the I399C>T had no significant effects on UGT1A9 mRNA levels and in vitro glucuronidation activities toward the two UGT1A9 substrates, flavopiridol and mycophenolic acid. Furthermore, another report has demonstrated that I399C>T had no influence on the pharmacokinetic parameters (such as AUC and C_{max}) of mycophenolic acid in 80 Japanese renal transplant recipients (Inoue et al., 2007). Thus, these latter two studies did suggest that the I399C>T polymorphism has no effect on UGT1A9 enzymatic activity. Note that, at least for Japanese populations, no study has reported that I399C>T affects UGT1A9 activity.

As for the influence of I399C>T on SN-38 glucuronidation, a possible enhancing effect has been suggested. Girard et al. (2006) have shown an increasing effect of I399C>T on SN-38 glucuronidation, and that this SNP did not show any close linkages with the *UGT1A1*28* or **60* allele ($r^2 < 0.06$). In addition, Sandanaraj et al. (2008) have reported that in 45 Asians consisting of Chinese (80%), Malay (18%), and others (2%), I399C/C patients had higher SN-38 AUC than C/T and T/T patients. Again, this SNP was not in LD with the *UGT1A1*28*, **6*, or **60* allele (r^2 were <0.09). Furthermore, association of I399T with increased SN-38G C_{max} has been observed even after stratified patients by *UGT1A1* genotypes, although the study sample size was small. These findings suggest that the I399T

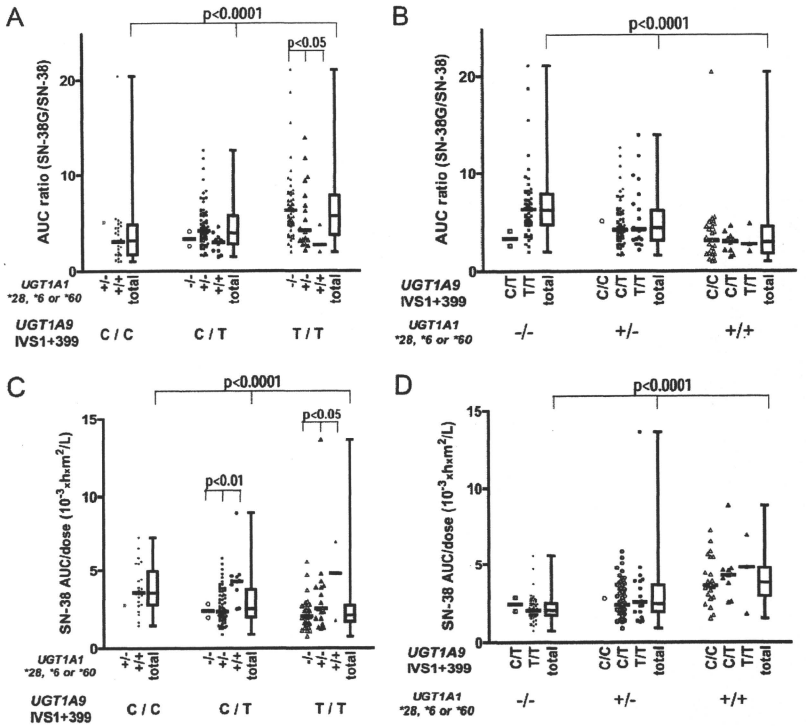

FIG. 2. Association analysis of *UGT1A9* IVS1+399 (I399)C>T with SN-38G/SN-38 AUC ratio (A and B) and SN-38 AUC/dose (C and D). A and C, I399 C/C, C/T, and T/T patients were further divided by the presence of *UGT1A1*28*, **6*, or **60* haplotypes: −/−, no *UGT1A1*28*, **6*, or **60*; −/+, heterozygotes for either *UGT1A1*28*, **6*, or **60*; +/+, homozygotes or compound heterozygotes for either *UGT1A1*28*, **6*, or **60*. B and D, *UGT1A1* −/−, −/+, and +/+ patients were further divided by I399 C/C, C/T, and T/T genotypes. Gene dose effects of I399C>T and the *UGT1A1* + haplotype were assessed by the Jonckheere-Terpstra test.

allele was associated with increased glucuronidation activity for SN-38 without linkages with the *UGT1A1* polymorphisms. Our data demonstrate that an increase in SN-38G/SN-38 AUC ratio (i.e., increased glucuronidation activity) was also found with I399C>T; however, after stratified patients by the *UGT1A1*6*, **28*, or **60* haplotypes (haplotype+) showing reduced SN-38 glucuronidation activity (Sai et al., 2004; Minami et al., 2007), any significant effect of the I399C>T was no longer observed. Thus, no direct effect of I399C>T on SN-38 glucuronidation was shown in the current study in Japanese populations. The discrepancy between our study and others might be derived from ethnic and/or population differences in haplotype distribution. In fact, in our Japanese population, 98% of the I399C alleles were linked with either *UGT1A1 *6*, **28*, or **60*, whereas 85% of the T alleles were linked with *UGT1A1*1*. On the other hand, in Sandanaraj's report (in Chinese + Malay), 84% of the I399C alleles were linked with *UGT1A1 *6*, **28*, or **60*, whereas only 67% of the T alleles were linked with *UGT1A1*1* (Sandanaraj et al., 2008).

In irinotecan therapies, genetic polymorphisms leading to increases in SN-38 AUC, which closely correlates with increased risk of severe neutropenia (Minami et al., 2007), are clinically important. The current study also demonstrated no significant influence of I399C>T on SN-38 AUC/dose after stratified patients by *UGT1A1* haplotypes. Consistent with this finding, no influence of this SNP was observed on the incidence of grade 3 or 4 neutropenia after irinotecan therapy in our population (data not shown). Recently, genetic testing of *UGT1A1*6* and **28*, which are related to severe neutropenia in Japanese populations, has been approved for clinical application in Japan. This study indicates that there is no clinical necessity for additional genotyping of I399C>T, at least in Japanese populations.

In conclusion of this study, the apparent influence of I399 (*UGT1A9* IVS1+399)C>T on SN-38 glucuronidation is attributable to its close association with *UGT1A1*6*, **28*, or **60* in the Japanese population. Furthermore, additional genotyping of I399C>T for personalized irinotecan therapy seems to be clinically irrelevant for Japanese populations.

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13. 消化管癌に対する化学療法の進歩

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動　向

2007年，胃癌の化学療法において，日本から重要な臨床研究結果が発表された．すなわち，1月のASCO（American Society of Clinical Oncology; 米国臨床腫瘍学会)-Gastrointestinal（G-I）Cancers SymposiumにおけるACTS-GC，および，6月のASCO annual meetingにおける，JCOG9912，SPIRITSという3つの第III相試験の結果の報告である．これらにより，本邦における胃癌治療の標準化の流れは一気に加速したように思える．また，大腸癌領域でも本邦においてはbevacizumabの認可がおりた画期的な年になった．ASCOではcetuximabの1次治療における有用性が検証され，分子標的治療薬は大腸癌治療において不可欠なものとなりつつある．本稿では，胃癌，大腸癌に対する化学療法の現況につき概説する．

A. 胃　癌

1. 術後補助化学療法

胃癌の術後補助化学療法については，手術単独を対象として進行再発胃癌に有効な多剤併用療法を用いた数々の比較臨床試験が行われてきたが，その有用性は確立していなかった．欧米においては術後補助化学療法＋放射線療法の有用性が報告されたが，標準術式が異なる本邦の胃癌患者に，これらの試験結果を外挿するのは適当ではないと考えられている．

国内外のmeta-analysisの報告[1-4]では手術単独群と比較し，術後補助化学療法の有意な予後改善効果が示唆されてきたが，本邦においてはJCOG（Japan Clinical Oncology Study Group）8801試験[5]においても，また引き続き実施されたJCOG9206－01，9206－02両試験[6,7]においても手術単独に対する術後補助化学療法の有用性は検証されなかった．このため，本邦では胃癌術後は経過観察のみとすることが標準的であった．

2007年のASCO G-I Cancers Symposiumにおいて国内の大規模比較試験（ACTS-GC）によりD2郭清を行い治癒切除を受けたStage II，IIIの症例に対するS-1の術後補助化学療法としての有用性が報告された[8]．すなわちStage II（ただし，T1を除く），III症例において術後補助化学療法としてS-1単剤を1年間内服した群の3年生存率は80.5%と手術単独の70.1%に比べ明らかに延長が認められた（表1）．さらにS-1内服群は毒性も非常に低く，内服コンプライアンスを保ちながら安全に施行できた．Grade 3以上の血液毒性はいずれも2%以下，Grade 3以上の非血液毒

表1　ACTS-GCの結果（文献8より改変）

	3年生存率	3年無再発生存率
手術単独群	70.1	60.1
S-1群	80.5	72.2
Stage II	90.7	83.7
Stage IIIa	77.4	69.1
Stage IIIb	64.3	49.9

性については食欲不振6%，嘔気3.7%，下痢3.1%といずれも低頻度であった．これにより，現時点でのStage II，III症例においての現在の標準治療はS-1の術後1年間の内服というコンセンサスが得られつつある．

a. 今後の方向性

ACTS-GCの結果ではStage IIの3年生存率はS-1群で90.7%と非常に良好であり，今後はS-1の内服期間（6カ月vs12カ月）などが検討課題となる．一方Stage IIIでは3年生存率はIIIa，IIIbでそれぞれ77.4%，63.4%と，いまだ十分とはいえない．2007年のASCOにおいて切除不能進行再発胃癌の標準治療とされたS1＋CDDPなどのcombination chemotherapyや術前補助化学療法などのさらなるアプローチにより治療成績の向上を目指していく必要がある．

2. 切除不能進行再発胃癌

治癒切除不能進行再発胃癌に対する化学療法のレジメンとしては，5-FU＋CDDP[9]，CPT-11＋CDDP[10]，ECF (Epirubicin＋CDDP＋5-FU)[11]，DCF (Docetaxel＋CDDP＋5-FU)[12] など様々なレジメンが報告されており，近年ではS-1単剤[13]やS-1を軸にした様々なcombination (S-1＋CDDP[14]，S-1＋CPT-11[15]，S-1＋Paclitaxel[16]，S-1＋Docetaxel[17] など）が有望であると報告されている．しかし，5-FU単独と比して明らかな生存期間の延長を得られたものはDCF以外になかった．JCOGではこの5-FU単独をcontrol armとして第II相試験で有望とされたCPT-11＋CDDPおよびS-1をtest armとした第III相試験（JCOG9912）を実施した．

a. JCOG9912試験

JCOG消化器がん内科グループでは，先のJCOG9205という試験において切除不能・再発胃癌に対する化学療法として5-FU持続静注療法をreference armと結論したため，今回も5-FU持続静注をコントロールとして，CPT-11＋CDDPとS-1の比較第III相試験（JCOG9912）が実施された[18]．primary endpointはoverall survival (OS) とし，CPT-11＋CDDPの5-FUに対する優越性，TS-1の5-FUに対する非劣性を証明することとした．secondary endpointはtime to treatment failure (TTF)，非入院生存期間，毒性，奏効率（ORR; RECISTに基づく）とされた．

704例が登録され680例が解析可能であり，最終結果は2007年のASCOにて発表された（表2）．奏効率，TTF，progression free survival (PFS)，OSすべてにおいてCPT-11＋CDDP群がよい傾向にあったが，5-FU群（MST10.8カ月）に対するCPT-11＋CDDP群（MST: 約12.3カ月）の優越性は証明されなかった（片側p＝0.055）．しかし，5-FU群に対するS-1群 (MST: 約11.4カ月) の非劣性は証明され（片側p＜0.001），毒性も軽微であったことが報告された．

b. S-1vsS-1＋CDDP（SPIRITS試験）

企業主導により切除不能・再発胃癌に対するS-1 vs S-1＋CDDPのS-1市販後臨床試験の最終結果も時を同じくして2007年のASCOにて発表された[19]．primary endpointはOS，secondary endpointはPFS，TTF，ORR，毒性であった．その結果，OSにおいてS-1群（MST: 11.0カ月）に対するS-1＋CDDP群（MST: 約13.0カ月）の優越性が有意差をもって証明された（p＝0.0366）．PFSも同様に4.0カ月 vs 6.0カ月とS-1＋CDDP群で有意に延長した（p＜0.0001）．グレード3/4の有害事象（S-1 vs S-1 CDDP）は

表2 JCOG 9912試験の結果（文献18より改変）

	N	Response Rate (%)	Median TTF (M)	Median PFS (M)	Median OS (M)	one-side p value
5-FU	234	9	2.3	2.9	10.8	－
CPT-11＋CDDP	236	38	3.7	4.8	12.3	0.055 (superior)
S-1	231	28	4.0	4.2	11.4	＜0.001 (non-inferior)

5-FU群: 5-FU 800mg/m^2 day 1～5, 4週ごと
CP群: CPT-11 70mg/m^2 day 1,15, CDDP 80mg/m2 day 1,4週ごと
TS-1群: TS-1 80mg/m^2 day 1～28, 6週ごと

白血球減少2% vs 12%, 好中球減少11% vs 40%, 貧血4% vs 26%, 嘔気1% vs 12%, 食欲不振6% vs 30%であった. なお, 治療関連死は認めなかった. 以上より, 進行胃癌に対するS-1＋CDDP療法は, S-1単独療法に比べて, 有効で忍容性にも優れていると報告された.

c. 今後の方向性

以上2つの切除不能・再発胃癌に対する第III相試験の結果から, S-1＋CDDP療法が, 切除不能進行再発胃癌の標準治療の有力な候補と考えられることになる. 真のglobal standardとなりうるかは, 現在欧米で進行中で, 本年報告される予定の, 5-FU＋CDDP (FP) とS-1＋CDDPを比較する大規模第III相試験 (FLAGS) の結果を待つことになる.

B. 大腸癌

1. oxaliplatin登場後の大腸癌化学療法

Grotheyらは主な第III相試験の検討において5-FU/LV, irinotecan, oxaliplatinの3剤が全治療期間内に使用された症例の割合と全生存期間が相関することを明らかにしており, 薬剤を変更しながら治療を継続することが生存期間の延長に結びつくとした[20]. その後の大腸癌化学療法におけるさらなるbreakthroughは, 分子標的治療薬の出現である. 2003年のASCOにおいて, 大腸癌領域において分子標的治療薬の臨床応用が初めて報告された.

一方, 5FU＋LVを含む各種併用療法において, 経口抗癌剤へ置換可能かどうかを検討する比較試験が行われ, 経口抗癌剤の位置づけが検討されている. 例えば, FOLFOX療法のinfusional 5FU/LVの部分をcapecitabineへ置換したXELOX療法は, 2006年ESMO (European Society for Medical Oncology; 欧州癌治療学会議) においてFOLFOXに対して非劣性が示されている (NO16966試験)[21]; また, oxaliplatinの蓄積性の神経毒性に対しても, 投与方法, 期間の模索がなされてきた. 2004年発表されたOPTIMOX1

表3 SPRITS試験の結果（文献19より改変）

	N	Response Rate (%)	Median TTF (M)	Median PFS (M)	Median OS (M)
S-1	150	31	3.9	4.0	11.0
S-1+CDDP	148	54	4.8	6.0	13.0
			p=0.009	p=<0.0001	p=0.037

S-1単独群: S-1 40～60mg/m^2 1日2回, day 1～28, 休薬day 29～42
S-1＋CDDP群: S-1 40～60mg/m^2 1日2回, day 1～21, 休薬day 22～35
CDDP 60mg/m^2 day 8

試験では神経毒性が強く出現する前にoxaliplatinを休薬し，一定期間の後に再導入するという“stop and go strategy”による毒性の減少が示された[22]．これに引き続き2006年，2007年のASCOではOPTIMOX2試験の結果が発表され，FOLFOX7（FOLFOX6のoxaliplatinを130mg/m^{2}に増量したregimen）を6サイクル施行後にsLV5FU2（FOLFOXからoxaliplatinを除いたregimen）を行うべきか，sLV5FU7による維持療法を行わずに休薬するchemotherapy free interval（CFI）をおくことが可能かについて検討がなされた[23]．primary endopointであるDDC(duration of disease control)は同等であったが，無増悪生存期間はCFI群で有意に短かった．全生存期間も有意差はないもののCFI群で短く，FOLFOX再導入後のRRおよびPFSは差がなかった．この結果は完全なCFIをおくことは勧められず，維持療法の必要性を示すものだと報告された．また，neuromodulatory agentsとして神経毒性を回避する目的でxaliprodenやCa/Mgなどいくつかの薬剤について臨床試験が行われているが，現在まで明らかな有効性を示したものはない．

2. 分子標的治療薬

a. bevacizumab

bevacizumab（avastin; 以下BV）剤は，血管内皮細胞増殖因子VEGF（vascular endotherial growth factor）に対するヒト化単クローン抗体である．IFL（irinotecan＋5-FU/LV）を対照群，IFL＋BV併用を試験群として初回化学療法例での比較検討（AVF2107g試験）が行われ，血管新生阻害剤として初めて生存期間を延長するという事実を示した[24]．続いて現在の標準治療の1つであるFOLFOX4とBVの併用療法の有用性が二次治療症例を対象としたランダム化第III相試験(E3200試験)[25]で示された．この結果を受け，現在FOLFOX＋BVまたはFOLFIRI＋BV併用療法が初回治療に対する標準治療と認識されている．有害事象は併用群において出血，血小板減少，蛋白尿，高血圧などの頻度が高く認められ，また，消化管穿孔が低頻度であるが併用群で多く認められている．

NO16966試験は前述のとおり，当初はXELOXのFOLFOX4に対する非劣性を検証する目的で開始されたが，AVF2107g試験の結果を受けてXELOXまたはFOLFOX4へのBV併用効果の検証も目的として2×2のfactorial designに変更，実施された2000人規模の大規模臨床試験である．PFSはBVなしvsあり＝8.0 vs 9.4カ月（p＝0.0023）とBVの上乗せ効果が検証された．しかし有害事象の検討では，消化管穿孔の頻度には差がなかったがGrade 3以上の高血圧はBV群で高く，有害事象による中止もBV群で高かった[21]．

b. cetuximab

上皮細胞増殖因子受容体EGFR（epidermal growth factor receptor）に対するマウス-ヒトキメラ単クローン抗体であるcetuximab（erbitux; 以下C225）も，2003年のASCOにおいてirinotecan不応大腸癌に対してC225単独とC225＋irinotecan併用群を比較した成績（BOND試験）[26]が報告され，奏効率や無増悪期間での優位性が検証された．主な有害事象は，キメラ抗体であるためinfusion reactionが認められること，にきび様の皮疹，爪の変形，肺臓炎などが報告されており，皮疹は効果と正の相関が認められている．

その後，主に二次治療以降で検討されていたC225の初回治療における有効性が第II相試験で報告され，なかでもFOLFOX4とC225の併用療法であるACROBAT試験では，奏効率81%，無増悪生存期間12.3カ月，また当初切除不能であった転移巣の切除が9例で可能となるなど非常に良好な成績が報告された[27]．また，FOLFIRI±C225およびFOLFOX±C225の2×2のの比較試

験の中間発表が2006年のASCOでなされ，C225あり：なしで奏効率は52%：32%とC225ありが有意に優れていた[28]．さらに2007年のASCO，AACR（American Association for Cancer Research; 米国癌研究会議）において一次治療として，CRYSTAL試験（FOLFIRI±C225）が，二次治療としてFOLFOX 抵抗例に対するEPIC試験（CPT-11±C225）が，また5FU，CPT-11およびoxaliplatinすべてに不応な症例に対してNCIC-CO. 17試験 (best supportive care vs C225) が報告された．CRYSTAL試験は登録数1000人規模の大規模第III相比較試験で，primary endpointはPFS，secondary endpointはORR，disease control rate（CR＋PR＋SD: DCR），OS，QOL（EORTC-QLQ-C30），安全性である．PFSはFOLFIRI/$^{+}$C225群8.9カ月，FOLFIRI群8.0カ月であり，HR 0.851［95%CI 0.726～0.998］と有意差を認めた（p＝0.0479）．ORRはFOLFIRI/$^{+}$C225群46.9%，FOLFIRI群38.7%で，DCRはFOLFIRI/C225群85.5%，FOLFIRI群84.3%といずれも併用群で優れた結果であった[29]．EPIC試験ではクロスオーバーデザインのためOSに差は認めなかったが，ORRとPFSは有意差をもって改善が認められた[30]．NCIC-CO. 17試験[31]では，OS，PFS，ORR全てにおいてC225群が有意に優れていた（表4）．いずれの試験においても併用群ではグレード3/4の下痢がやや多くみられ，皮疹の増加がみられた．しかし，皮疹は予想された範囲内であった．また，皮疹のグレード別にPFSをみると皮疹のグレードとPFSには正の相関がみられた．以上の結果より一次治療，二次治療，refractoryいずれにおいてもC225の上乗せ効果が認められたといえる．さらに，C225/BVの併用療法も報告されてきている．Saltzらはirinotecan不応性の切除不能再発結腸直腸癌74例を対象に，C225＋BV±irinotecan併用療法を実施した（BOND2試験）[32]．C225＋BV（CB）群およびC225＋BV＋CPT-11（CBI）群の奏効率はそれぞれ，23%，38%という結果で，同様にirinotecan不応例に対しC225単独とC225＋irinotecan併用群を比較したBOND1試験[33]における奏効率（11%vs. 23%）を上回る結果が得られた．これにより，BVはirinotecan不応例に対し，C225単剤またはC225/irinotecan併用群の有効性をさらに高める可能性が示唆された．さらに現在BV不応例に対し，CB/CBI群の有用性の検討がなされている（BOND3試験）．

c. panitumumab（ABX-EGF）

完全ヒト型抗EGFR抗体であるpanitumumab（ABX-EGF）は，キメラ抗体であるC225に比べ，infusion reactionなどの有害事象の頻度が少ないと考えられている．irinotecanおよびoxaliplatinに不応となり有効な治療法がない大腸癌患

表4　cetuximabの海外第III相臨床試験の結果（文献28，29，30より改変）

Study No.	Regimen	N	RR	p	PFS	p	OS	p
CRYSTAL	FOLFIRI±	599	38.7		8.0	0.851	-	
	cetuximab	599	46.9	0.0038	8.9	0.0479	-	
EPIC	CPT-11±	650	4.2		2.6	0.692	9.99	0.975
	cetuximab	648	16.4	＜0.0001	4.0	＜0.0001	10.71	0.7115
NCIC-Co. 17	BSC±	285	0		1.8	0.68	4.6	0.77
	cetuximab	287	6.6	＜0.0001	1.9	＜0.0001	6.1	0.0046

cetuximab: 400mg/m^2 Initial dose week 1 of cycle1 followed by 250mg/m^2 weekly starting week 2
FOLFIRI: Iri 180mg/m^2＋5FU（400mg/m^2 bolus＋2400mg/m^2 46hrs continuous infusion＋FA）q2wks
Irinitecan: 350mg/m^2 q3wks

者を対象にABX-EGF単剤とBSCとの比較試験が行われ，無再発生存期間においてABX-EGF群が優れていたことが報告された[34]．現在，同剤とFOLFOXやFOLFIRI，BVなどとの併用療法の検討も行われている．

3. 現在の治療体系

現在NCI（National Cancer Institute: 米国国立癌研究所）やNCCN（National Comprehensive Cancer Network: 全米癌総合ネットワーク）などにおいてこれらの臨床試験の結果に基づいた治療法選択のガイドラインが公表されており[35,36]腫瘍専門医がこの情報をもとに治療法を選択するようになっている．それによると，一次治療としてFOLFOX（またはCapeOx）＋BVを行った場合，二次治療はFOLFIRIもしくはirinotecan単剤±C225，そして三次治療でC225±irinotecan（2次治療でC225を用いなかった場合）あるいはABX-EGFとなっている．または，一次治療でFOLFIRI＋BVを選択した場合は，二次治療，三次治療でFOLFOX（またはCapeOx）もしくはC225±irinotecanあるいはABX-EGFを用いることが推奨されている．

むすび

胃癌領域においては2007年，本邦より標準治療を決定づけるような重要な臨床試験の結果が報告された．今後も世界をリードし続けるようなqualityの高い試験を継続し情報を発信していくことが求められる．大腸癌領域においては，欧米との格差はいまだ歴然としているが，global試験への参加などを通じて，積極的に世界へアピールしていくことが肝要であろう．

combination chemotherapyの有用性の検証や分子標的治療薬の登場で消化器癌の化学療法は治療法選択の幅がさらに広がりつつあるが，分子標的治療薬では今までの殺細胞性薬剤にみられなかった毒性の発現も認められており，QOLを考慮した上での適切な薬剤選択と治療継続の判断を行うことがますます重要となっている．国際的標準治療の変化を常にフォローしながら，最善の治療法を患者に提供することができる臨床能力が個々の腫瘍専門医に求められている．

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