Against this, however, treatment options for metastatic CRC patients with disease refractory to all three antitumor drugs are limited.

The addition of bevacizumab, a recombinant humanized monoclonal antibody, which targets vascular endothelial growth factor, to front- or second-line chemotherapy has improved progression-free survival (PFS) and OS (5). However, the clinical benefits of bevacizumab in third-line therapy have yet to be shown. In this regard, the National Comprehensive Cancer Network (NCCN) clinical practice guidelines (6,7) do not recommend bevacizumab as third-line therapy.

Cetuximab, a chimeric antibody of the IgG1 subclass, blocks the binding of the epidermal growth factor (EGF) and transforming growth factor alpha to the EGF receptor (EGFR) and inhibits ligand-induced activation of this receptor tyrosine kinase (8). Cetuximab has demonstrated antitumor activity in both *in vivo* and *in vitro* study (8). In addition, this agent not only enhanced the effects of irinotecan and radiotherapy (9) but also showed the ability to reverse resistance to irinotecan in preclinical studies (10).

Two previous studies have investigated the use of combination therapy of cetuximab plus irinotecan in subjects with irinotecan-refractory disease; the first, a single-arm Phase II trial, reported a response rate (RR) of 17% in 121 patients (11), the second, a randomized Phase II study of single agent cetuximab or cetuximab plus irinotecan, the BOND trial, reported a RR of 10.8% (111 subjects) versus 22.9% (218), respectively (12). Moreover, response in the combination arm in those who had received oxaliplatin was 22.2%. These results suggest that combination therapy of cetuximab plus irinotecan may benefit subjects refractory to irinotecan-based chemotherapy who have received oxaliplatin-based chemotherapies.

At the time the present study was undertaken, neither cetuximab nor bevacizumab was approved in Japan. Standard management of CRC instead consisted of the use of irinotecan, oxaliplatin or fluoropyrimidines, with no other standard options available after progression on these drugs. We therefore conducted a multicenter phase II study of cetuximab plus irinotecan in metastatic CRC refractory to irinotecan, oxaliplatin and fluoropyrimidines to evaluate the efficacy and safety of this combination.

PATIENTS AND METHODS

Study Design and Patient Eligibility

The study was designed as a phase II, non-randomized, open-label, multicenter trial. Eligibility requirements included histologically confirmed, metastatic CRC that was surgically unresectable, as well as immunohistochemical evidence of EGFR expression measured semiquantitatively (>0 on a scale of 0, 1+, 2+ or 3+) at a single reference laboratory (SRL Medisearch, Inc.). Patients were required to have received irinotecan-based chemotherapy at a weekly irinotecan dose of $\geq$60 mg/m^2 or every 2 weeks at $\geq$100 mg/m^2, both defined as final doses, for at least 6 weeks or more. They were also required to have radiographically documented evidence of disease progression during this previous chemotherapy or within 3 months following the last dose of irinotecan. Further, patients were required to have received and failed fluoropyrimidine- and oxaliplatin-based chemotherapies. For this requirement, failure was defined as progression of disease (clinical or radiological) while receiving the previous oxaliplatin-based chemotherapy or within 6 months following the last treatment of an adjuvant therapy, or intolerance to the oxaliplatin-based chemotherapy. Regarding intolerance, this was defined as discontinuation due to allergic reaction, persistent neurotoxicity or delayed recovery from other toxicity that prevented re-treatment.

Other eligibility criteria included age 20 to less than 75 years, an Eastern Cooperative Oncology Group (ECOG) performance status (PS) score of 0–2, life expectancy of at least 2 months, and adequate organ function (absolute neutrophil count $\geq$1500/mm^3, platelet count $\geq$100 000/mm^3, hemoglobin $\geq$9 g/dl, aspartate aminotransferase and alanine aminotransferase $\leq$2.5 times the upper limit of normal range, serum total bilirubin $\leq$1.5 times the upper limit of normal range and serum creatinine $\leq$1.5 mg/dl). Minimum treatment-free periods between the end of prior therapy and day of registration were 6 weeks for radiation therapy, 4 weeks for major surgery and 4 weeks for chemotherapy. At least one unidimensionally measurable target lesion by Response Evaluation Criteria in Solid Tumors (RECIST) criteria was required. Patients who previously received EGF signal transduction inhibitors or EGFR-targeting therapy were not eligible.

This protocol was reviewed and approved by the institutional review board of each participating center, and all patients gave written informed consent before participation.

Dosage and Drug Administration

The initial dose of cetuximab was administered as a single 2-h intravenous infusion at 400 mg/m^2 followed by weekly 1-h infusions of 250 mg/m^2. All patients were premedicated with an H1 histamine antagonist (e.g. diphenhydramine hydrochloride 50 mg po). Irinotecan was administered under the same schedule as the previous irinotecan-based therapy, namely either weekly at a dose of 100 mg/m^2 on Days 1, 8, 15 and 22, repeated every 6 weeks; or every 2 weeks at 150 mg/m^2 on Days 1, 15 and 29, repeated every 7 weeks.

Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0.). If a patient experienced Grade 3 or worse skin toxicity, cetuximab therapy was withheld for up to two consecutive infusions with no subsequent change in dose level. If the toxicity resolved to Grade 2 or less by the following treatment period, treatment was resumed. With the second and third occurrences of Grade 3 or worse skin toxicity, cetuximab therapy was delayed again for up to two consecutive weeks with concomitant dose reductions to 200 and 150 mg/m^2, respectively. Treatments were discontinued if

more than two consecutive infusions were withheld or if there was a fourth occurrence of Grade 3 or worse skin toxicity. If a patient experienced a Grade 3 or worse hypersensitivity reaction, treatments were immediately discontinued. If a patient experienced a Grade 2 hypersensitivity reaction, cetuximab infusion was stopped, and if the reaction resolved to Grade 1 or less, it was resumed at half the previous infusion rate. Dose modification and treatment alterations for irinotecan were performed in accordance with hematological and non-hematological toxicities. If a patient experienced a Grade 4 thrombocytopenia or Grade 3 or worse neuropathy, irinotecan therapy was stopped. If a patient experienced a Grade 3 or worse febrile neutropenia, thrombocytopenia, or non-hematological toxicity, or Grade 4 neutropenia, irinotecan dose was reduced by one dose level.

Evaluation of Patients

Medical history, physical examination, laboratory test assessments and safety assessments were performed once before starting treatment and weekly thereafter. Chest X-ray was taken every 6 weeks.

Tumor measurement was performed within 4 weeks prior to starting administration of study therapy. Response was evaluated every 6 weeks thereafter according to the RECIST criteria. All responses were confirmed by an independent review committee.

Statistical Analysis

A sample size of 38 response-evaluable subjects was established based on expected and threshold RRs of 20 and 5%, respectively, under conditions of $\alpha = 0.05$ (one-tailed) if the RR was lower than the threshold RR, and $\beta \geq 0.1$ if higher. The primary endpoint was the RR. If 38 subjects were response-evaluable, the null hypothesis would be rejected if at least five responses were observed. A patient who received at least one dose of study therapy was considered evaluable for response. Secondary endpoints, including duration of response and time to progression (TTP), were estimated using the Kaplan–Meier method.

TTP was defined as the period from the date treatment was started to the first observation of disease progression or to death from any cause before the confirmation of disease progression or after the most recent tumor assessment.

OS time was determined from the date of first administration of chemotherapy to the date of death or the last confirmation of survival. Duration of response was considered the period between the date of first confirmation of response and the date of documented disease progression, or the last confirmation of response. Comparisons among different subgroups of patients were performed using the log-rank test, and RR was the Fisher exact test. All analyses were conducted using SAS software (version 8; SAS Institute, Cary, NC, USA).

RESULTS

Patient Characteristics

Forty-four of 46 centrally screened patients demonstrated detectable expression of EGFR. Of these 44, 39 consecutive patients were enrolled between October 2005 and February 2006. All patients received combination treatment with irinotecan and cetuximab. Patient characteristics are summarized in Table 1 and show a median age of 58 years, male predominance and good performance status. More than 60% ($n = 25$) of patients had received three or more regimens before entry.

All patients had been refractory to irinotecan-based regimens and had received oxaliplatin and fluoropyrimidine before participation in the study. The median duration of

Table 1. Patient characteristics

Patient characteristics	Number of patients (%)
Total number of patients	39
Sex	
Male	27 (69.2)
Female	12 (30.8)
Age	
Median	58.0
Range	32–72
Performance status (ECOG)	
PS 0	26 (66.7)
PS 1	13 (33.3)
Primary tumor site	
Colon	18 (46.2)
Rectum	21 (53.8)
Sites of metastases	
Liver	30 (76.9)
Lung	27 (69.2)
Lymph nodes	17 (43.6)
Peritoneum	5 (12.8)
Rectum	2 (5.1)
Other	6 (15.4)
Number of prior chemotherapy	
2	14 (35.9)
3	11 (28.2)
4	6 (15.4)
>5	8 (20.5)
EGFR expression	
1+	29 (74.4)
2+	10 (25.6)

ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor.

prior irinotecan-based therapy was 24 weeks (range 6–52 weeks). Responses to irinotecan-based regimen were 2.6% complete response (CR), 23.1% partial response (PR), 41% stable disease (SD) and 30.8% progression of disease (PD). Median time from the last pre-study irinotecan dose to the initiation of cetuximab therapy was 7.2 months, with a range of 1.0–18.3 months. Thirty-five patients (89.7%) discontinued oxaliplatin-based regimens for disease progression, with a median of 2.1 months after last treatment. Response to the most recent oxaliplatin-based regimen was 0% CR, 5.1% PR, 46.2% SD and 46.2% PD.

Among other characteristics, 12 patients (30.8%) had received adjuvant chemotherapy; 34 (87.2%) had undergone surgical resection of the primary tumor; 20 (51.3%) had recurrent disease; and 19 (48.7%) had advanced disease. Two patients had received radiotherapy for CRC.

Efficacy

All patients enrolled were considered evaluable for efficacy (Table 2). An independent review committee determined that 12 patients (30.8%; 95% CI, 17.0–47.6) achieved PRs, and 25 (64.1%; 95% CI, 47.2–78.8) achieved either PRs or SDs (disease control). Median duration of response was 5.4 months (95% CI, 3.9–6.4), and median time to response was 1.4 months (range 1.3–5.3). Median TTP was 4.1 months (95% CI, 2.7–5.1) (Fig. 1). With a median follow-up of 14.4 months, the median OS was 8.8 months (95% CI, 5.9–12.8) (Fig. 2) (Table 2).

No association with response was seen for either age, sex, performance status, number of prior chemotherapy regimens, primary tumor site or degree of EGFR immunostaining. In contrast, RR was significantly higher in patients who had achieved a response in a prior irinotecan regimen than in those who had not [($P = 0.04$ Fisher exact test, 60.0% (95% CI, 26.2–87.8) versus 20.7% (95% CI, 10.2–48.4)]. Moreover, median OS of patients who had achieved a response in a prior irinotecan regimen was slightly longer than that of those who had not, albeit without significance (9.9 versus 8.8 months, $P = 0.92$; log-rank test).

The presence and severity of rash did not correlate with objective response (RR with Grade 0–1 versus Grade 2–3: 31.6 versus 30.0%, $P = 0.73$; Fisher exact test).

Table 2. Efficacy

Best response	Patients ($N = 39$) [No. (%)]
CR	0 (0)
PR	12 (30.8)
SD	13 (33.3)
PD	14 (35.9)
Response	12 (30.8)
95%CI	17.0–47.6
Disease control	25 (64.1)
95%CI	47.2–78.8
Median TTP	4.1 months
95%CI	2.7–5.1
Median OS	8.8 months
95%CI	5.9–12.8

CR, complete response; PR, partial response; SD, stable disease; PD, progression of disease; CI, confidence interval; TTP, time to progression; OS, overall survival.

Adverse Events

Major adverse events are shown in Table 3. Overall, hematological toxicities were generally well tolerated, with Grade 3 or worse neutropenia observed in 23.1% of patients ($n = 9$). Febrile neutropenia was not observed. The incidence of Grade 3 or worse neutropenia was higher in patients receiving irinotecan every 2 weeks than in those on weekly regimens (27.6 versus 10.0%).

Among other adverse events, non-hematological toxicities were also mild, with Grade 3 or worse diarrhea and anorexia observed in seven (17.9%) and six patients (15.4%), respectively. An acne-like rash, which is characteristic of treatment with cetuximab and other EGFR-targeted therapies and included acne, rash, dry skin, pruritus, acneiform dermatitis and papular rash, was observed in 38 patients (97.4%). Median time to the appearance of cetuximab-related acne-like rash was 7.0 days (range 1–31), while the median cumulative dose of cetuximab until appearance was 400 mg/m^2 (range 400–1400) and duration of appearance was 121.5 days. Two patients (5.1%) experienced Grade 3 acne, with a duration of appearance of 10 and 15 days, respectively. All patients received topical treatment and 19 (51.4%) received oral antibiotic drugs, including minocycline. Median duration from the appearance of an acne-like rash to the start of antibiotic drugs, including topical treatment and minocycline, was 5.7 days. No patient experienced allergic reactions leading to the cessation of therapy. One patient discontinued treatment due to Grade 1 lung fibrosis, a condition that has never shown exacerbation without medication.

Duration of Treatment and Dose Intensity of Cetuximab and Irinotecan

The median duration of cetuximab treatment was 18 weeks (range 6–50 weeks), with a median of 16 infusions per patient (range 4–49 infusions). No patient required a dose reduction. Median dose intensity was 232 mg/m^2 per week (range 150–254 mg/m^2); 24 patients received doses at more than 90% of relative dose intensity, nine within 80–90% and six within 60–80%.

Fourteen patients (35.9%) required a dose reduction of irinotecan, primarily due to diarrhea (seven patients) and neutropenia (two patients). The median duration of treatment in all patients was 15 weeks (range 2–49), with a median of seven infusions per patient (range 2–30 infusions). Median

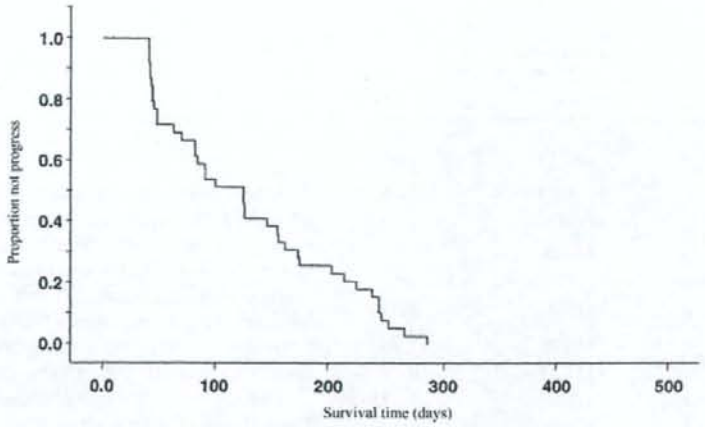

Figure 1. Kaplan–Meier survival plots for Time to Progression (TTP) in metastatic colorectal cancer (CRC) treated with certuximab plus irinotecan.

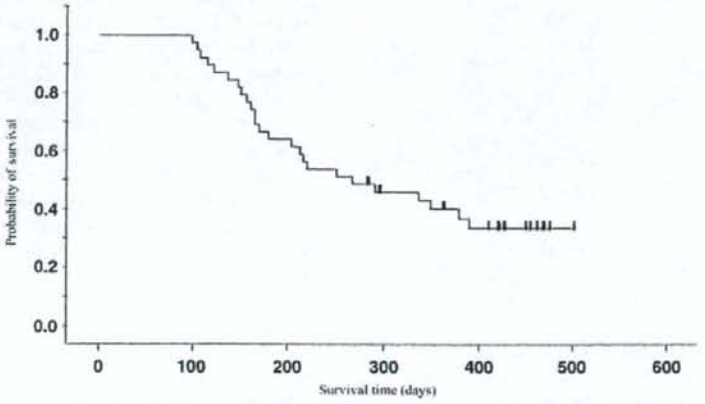

Figure 2. Kaplan–Meier survival plots for overall survival (OS) in metastatic CRC treated with certuximab plus irinotecan.

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.

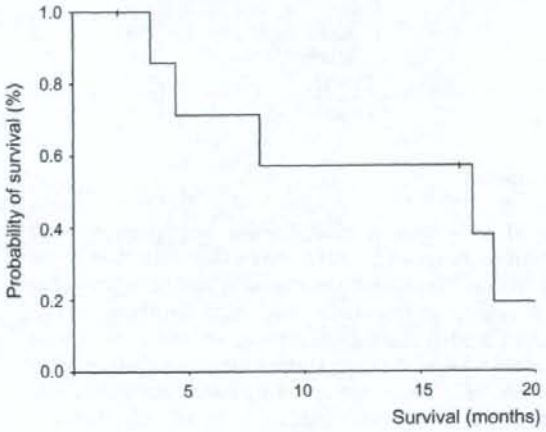

Fig. 1. Overall survival

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.

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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
Alelle 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

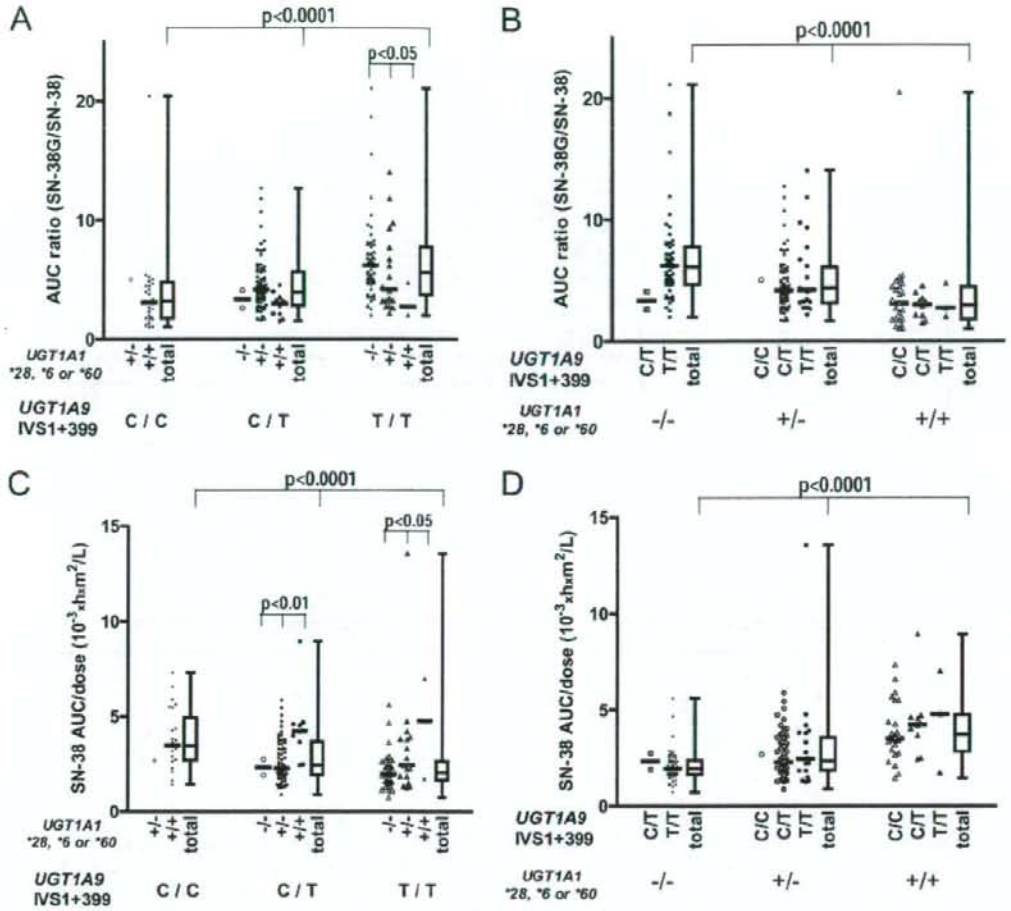

FIG. 2. Association analysis of *UGT1A9* IVS1+399 (I399)C>T with SN-38G/SN-38 AUC ratio (A and B) and SN-38 AUC/dose (C and D). A and C, I399 C/C, C/T, and T/T patients were further divided by the presence of *UGT1A1*28*, **6*, or **60* haplotypes: −/−, no *UGT1A1*28*, **6*, or **60*; −/+, heterozygotes 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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key words　gastric cancer, colorectal cancer, S-1, bevacizumab, cetuximab, panitumumab

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

Grotey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