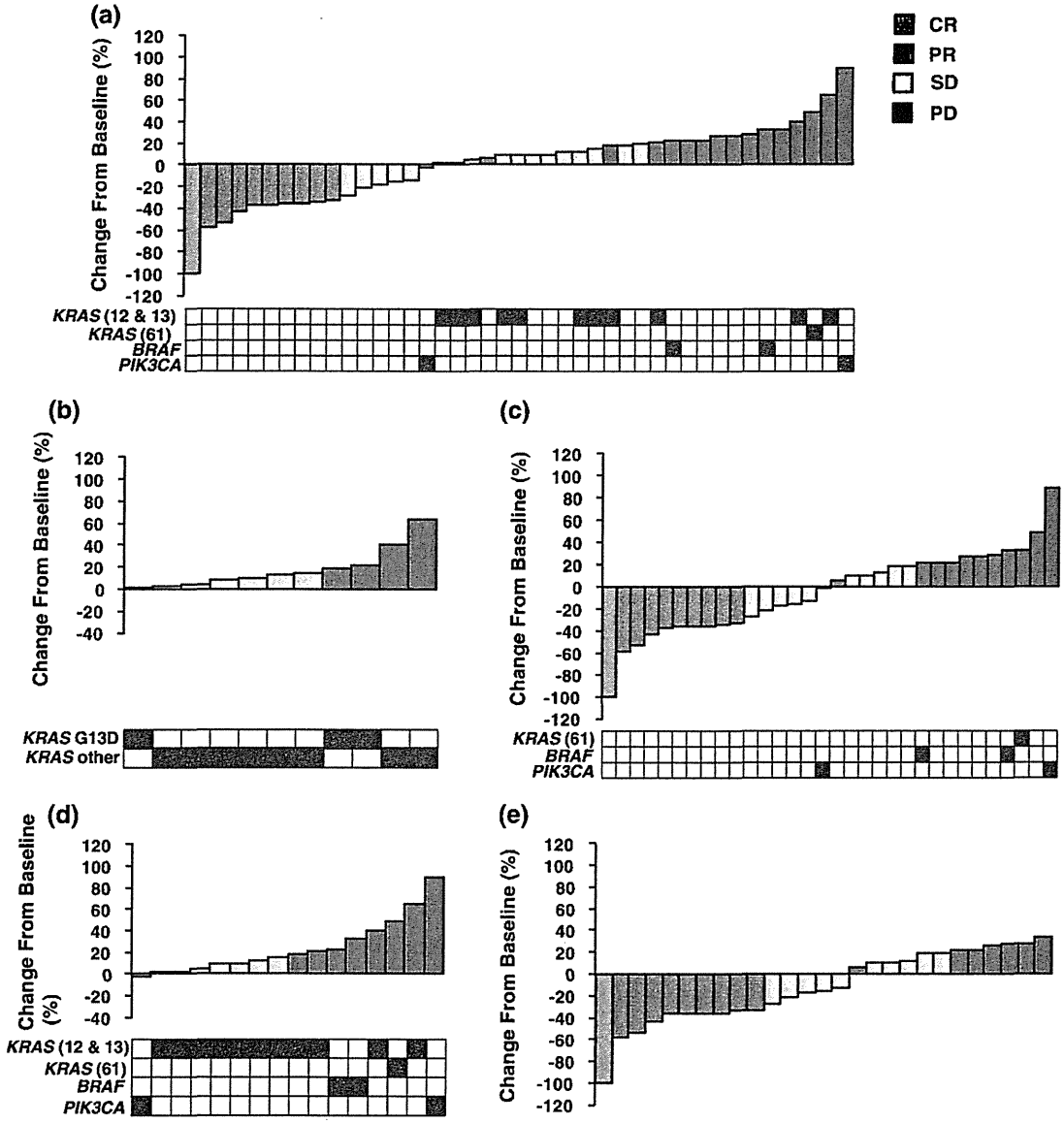

Fig. 2 *Waterfall plots* showing maximal reduction of target lesions based on *KRAS*, *BRAF*, and *PIK3CA* mutational status in mCRC patients treated with cetuximab. **a** All patients. **b** Patients with mutant *KRAS* (codons 12, 13). **c** Patients with wild-type *KRAS* (codons 12, 13). **d** Patients with any mutant *KRAS* (codons 12, 13, 61), *BRAF*, and *PIK3CA*. **e** Patients with all wild-type *KRAS* (codons 12, 13, 61), *BRAF*, and *PIK3CA*

In this study, the RR of cetuximab plus irinotecan was 32.3 %; the RR of cetuximab monotherapy was 8.3 % in the third or additional lines of treatment for mCRC. This efficacy was comparable with the data of 206 patients in the third-line subgroup in the BOND study (RR was 22.2 % for cetuximab plus irinotecan and 8.5 % for cetuximab monotherapy) [28] or the NCIC-CTG Co. 17 study (RR was 8.1 % for cetuximab monotherapy) [8]. The toxicity profiles were also consistent with those observed in these studies. Therefore, we conclude that both efficacy and safety of cetuximab treatment for chemotherapy-refractory patients are similar between Japanese and Caucasians.

In conclusion, the results of this study confirmed that cetuximab-based treatment is effective and well tolerated in patients with wild-type *KRAS* who have failed prior chemotherapy including irinotecan, oxaliplatin, and fluoropyrimidine in Japanese as in Caucasians. These results indicated the clinical relevance of *KRAS* mutations in predicting the efficacy of cetuximab-based treatment in Asian patients with mCRC. Moreover, our data also indicated that mutation analysis of *KRAS* codons 61, *BRAF*,

and *PIK3CA* contributes to improving the selection of candidate patients who are most likely to benefit from anti-EGFR mAbs.

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Safety Verification Trials of mFOLFIRI and Sequential Irinotecan + Bevacizumab as First- or Second-Line Therapies for Metastatic Colorectal Cancer in Japanese Patients

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Key Words
Advanced colorectal cancer · Irinotecan · Bevacizumab · S-1 · Randomized trial

Abstract
Objective: S-1 is effective in sequential combination with irinotecan (IRIS) in treating metastatic colorectal cancer. We conducted a randomized phase II trial of modified leucovorin, fluorouracil and irinotecan (mFOLFIRI) + bevacizumab and sequential IRIS + bevacizumab as first- or second-line therapies. ***Methods:*** Sixty metastatic colorectal cancer patients were randomly assigned to receive mFOLFIRI + bevacizumab or sequential IRIS + bevacizumab (7.5 mg/kg of bevacizumab and 150 mg/m^2 of irinitecan, and 80 mg/m^2/day of S-1 orally from day 3 until day 16 as a 3-week course). The primary endpoint was the safety of each method until week 12, with the secondary endpoint being the comparison of the safety and efficacy of the two methods. ***Results:*** The safety of the two treatments was comparable, except that G3 anorexia and diarrhoea were less frequent with sequential IRIS + bevacizumab. The overall response rate was 62% [95% confidence interval (CI) 40.1–79.8] versus 72% (95% CI 50.6–86.2), and progression-free survival was 324 days (95% CI 247–475) versus 345 days (95% CI 312–594) with mFOLFIRI + bevacizumab versus IRIS + bevacizumab, respectively. ***Conclusion:*** Sequential IRIS + bevacizumab is a safe and effective method of systemic chemotherapy against metastatic colorectal cancer and is compatible with mFOLFIRI + bevacizumab.

Introduction

Over the past 10 years, as a result of multidisciplinary therapies including systemic chemotherapy, there has been a dramatic improvement in the success of treat-

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ments against unresectable and/or recurrent colorectal cancer [1]. Particularly, based on the results of several clinical trials, bevacizumab was shown to extend progression-free survival (PFS) when used in combination with other chemotherapies including irinotecan, fluorouracil and leucovorin [2], leucovorin, fluorouracil and oxaliplatin (FOLFOX) [3], leucovorin, fluorouracil and irinotecan (FOLFIRI) [4], and 5-fluorouracil and leucovorin (5-FU/LV) [5]. These results are further supported by large-scale observational studies [6, 7]; however, in standard chemotherapy treatments, as often represented by either FOLFOX or FOLFIRI, placement of a peripherally inserted central venous port (CV port) is required for continuous 5-FU infusion. The usage of CV ports can cause complications, including infections and thrombosis, resulting in decreasing the patient's quality of life [8, 9].

In consideration of these factors, chemotherapy regimens using oral fluoropyrimidines rather than continuous 5-FU infusion must be developed. The CapeOX regimen, which uses capecitabine, an oral fluoropyrimidine pro-drug of 5-FU rather than 5-FU/LV, plus oxaliplatin, has identical therapeutic effects to FOLFOX. Favourable results were also observed when used in combination with bevacizumab [10]. However, because of severe gastrointestinal toxicity associated with capecitabine in combination with irinotecan (CapeIRI or XELIRI), an effective alternative treatment to FOLFIRI has yet to be developed [4].

S-1 is a combination of tegafur, a pro-drug of 5-FU that consists of oral fluoropyrimidines, gimeracil (5-chloro-2,4-dihydoroxypyridine) and oteracil (potassium oxonate) at a molar ratio of 1:0.4:1 [11]. Gimeracil has a reversible competitive inhibitory effect on dihydropyrimidine dehydrogenase, a rate-limiting enzyme involved in the metabolic degradation of 5-FU. Oteracil reduces gastrointestinal toxicity and is effective against a wide range of carcinomas. Against metastatic colorectal cancer, S-1 showed a response rate of 39.5%, a PFS of 5.4 months and an overall survival time of 11.9 months when used as a monotherapy [12]. Because S-1 is expected to replace 5-FU/LV, there have been several prospective clinical trials in Japan using S-1 in combination with oxaliplatin (L-OHP or SOX) [13]. Clinical trials of S-1 combined with irinotecan (IRIS) were also conducted with various schedules or dosage regimens [14–16]. Among these, Yoshioka et al. [15] conducted phase I/II trials of sequential IRIS and the combined treatment of staggered irinotecan and S-1. These clinical trials were performed in order to avoid decreased therapeutic effects and increased toxicities caused by the inhibitory effect of 5-FU and its metabolites on the bioactivation of SN-38 from irinotecan [17, 18]. The authors reported on how this treatment regimen effectively avoided toxicity and rivaled the efficacy of previous FOLFIRI treatments; however, because the introduction of molecular targeted drugs in Japan was delayed, no studies were performed on the safety and efficacy of sequential IRIS in combination with bevacizumab. Thus, we report on the respective safety of sequential IRIS + bevacizumab and modified FOLFIRI (mFOLFIRI) + bevacizumab therapies against unresectable colorectal cancer. A secondary comparative study on the safety and efficacy of both therapies was also performed.

Patients and Methods

Patient Eligibility

The eligibility criteria were as follows: (1) patients histologically diagnosed with colorectal cancer; (2) patients with either an unresectable primary tumour or distal metastatic tumours; (3) an Eastern Cooperative Oncology Group performance status of 0 or 1; (4) the previous chemotherapy regimen had to be ≤1; (5) patients of post-operative adjuvant chemotherapy >6 months since last administration of drugs; (6) in the case of second-line therapy, first-line therapy had to be FOLFOX treatment; (7) internal organ function maintained, i.e. white blood cell count of 3,500–12,000/μl, platelet count ≥100,000/μl, aspartate aminotransferase (AST) ≤100 IU/l, alanine aminotransferase (ALT) ≤100 IU/l, total bilirubin ≤ 1.5 mg/dl, serum creatinine ≤1.2 mg/dl, serum creatinine clearance as estimated by Cockcroft-Gault equation ≥50 ml/min; (8) survival expected to be at least ≥3 months; and (9) written informed consent obtained from the patient for trial participation.

Exclusion criteria were as follows: (1) a history of abdominal irradiation; (2) any complications, such as intestinal paralysis, intestinal obstruction, poorly controlled diabetes, poorly controlled hypertension, unstable angina, hepatic cirrhosis, interstitial pneumonia, pulmonary fibrosis or severe pulmonary emphysema; (3) body cavity fluid retention requiring treatment; (4) poorly controlled peptic ulcerations; (5) concomitant gastrointestinal perforation or a history of perforation within 1 year prior to registration; (6) brain tumours or cerebral metastases confirmed on imaging; (7) concomitant symptoms of cerebrovascular nerve damage or any type of cardiac disease requiring treatment; (8) surgical treatment within 4 weeks prior to registration; (9) a bleeding tendency, coagulation disorder or excessive clotting factors; (10) awaiting or on treatment for chronic inflammatory disease such as rheumatoid arthritis, with any drugs that inhibit platelet function (aspirin or non-steroidal anti-inflammatory drugs); (11) women who are pregnant, may be pregnant, wish to become pregnant or are lactating; (12) men who wish their partner to become pregnant; (13) patients using irinotecan as post-operative adjuvant chemotherapy.

Treatment Methods

In the sequential IRIS + bevacizumab treatment regimen, on day 1, 7.5 mg/kg of bevacizumab was administered for >30 min, and 150 mg/m^2 of irinotecan was administered continuously for >90 min. Then, for the 2-week period from days 3 to 16, divided doses of S-1 were administered twice daily. The dosage of S-1 was as follows: body surface area (BSA) <1.25 m^2, 80 mg/day; BSA 1.25–1.5 m^2, 100 mg/day, and BSA >1.5 m^2, 120 mg/day as a 3-week course. Dosage for the mFOLFIRI + bevacizumab treatment regimen was as follows: 5 mg/kg of bevacizumab, 150 mg/m^2 of irinotecan, 200 mg/m^2 of L-leucovorin, 400 mg/m^2 of 5-FU by rapid intravenous infusion on day 1, and 2,400 mg/m^2 of 5-FU for 46 h by continuous intravenous infusion as a 2-week course. The treatment protocol period was set at 12 weeks in both groups, and treatment was continued until the criteria for discontinuation of the trial were met.

The criteria for commencement of treatment in each course were as follows: white blood cell count ≥3,000/μl, platelet count ≥75,000/μl (mFOLFIRI + bevacizumab) or ≥100,000/μl (IRIS + bevacizumab), AST ≤100 IU/l, ALT ≤100 IU/l, total bilirubin ≤1.5 mg/dl, and serum creatinine ≤1.2 mg/dl. In addition, diarrhoea of grade 0 and improvement in any other non-haematologic toxicity (excluding constipation, loss of appetite, loss of hair, chromatosis and dysgeusia) of grade ≤1 was required. In patients where the criteria for commencement of treatment were not met, treatment was delayed until all necessary requirements were completely satisfied. Treatment was discontinued in those patients where the criteria for commencement of treatment were not met even after a delay of ≥3 weeks.

The criteria common to both groups for discontinuation of bevacizumab treatment were as follows: (1) any grade of haemoptysis, gastrointestinal perforation, reversible leucoencephalopathy syndrome; (2) grade ≥3 thromboembolism, haemorrhage or hypersensitivity reaction, and (3) grade 4 proteinuria or hypertension. In patients with grade 2 haemorrhage, treatment was withdrawn until improvement to grade 0, and treatment was discontinued in patients where grade 2 haemorrhage recurred. Treatment was discontinued in patients with grade 3 hypertension that could not be controlled by medication. Treatment was withdrawn in the following situation: patients with grade 2 or 3 proteinuria until proteinuria was ≤2 g as determined by 24-hour urine collection analyses, with grade 3 or 4 liver dysfunction until improvement to either grade 1 or baseline, and in instances of recurrence.

In the IRIS group, S-1 administration was stopped if any of the following adverse effects occurred during the course: (1) grade ≥3 leucopenia or neutropenia in addition to other grade ≥3 non-haematological toxicity, until patient recovery; (2) grade ≥2 thrombocytopenia, diarrhoea, stomatitis, nausea or vomiting; (3) serum creatinine ≥1.5× the upper limit of normal, and (4) AST or ALT ≥100 IU/l. Any patients exhibiting grade ≥4 leucopenia or neutropenia, grade ≥3 thrombocytopenia, diarrhoea, stomatitis, nausea or vomiting, non-haematological toxicity, or AST or ALT ≥200 IU/l during the study were administered a lower dosage of IRIS in the next course of treatment. The low dosage of S-1 (level 1) was 50 mg/day for BSA <1.25 m^2, 80 mg/day for BSA 1.25–1.50 m^2, and 100 mg/day for BSA >1.5 m^2. For irinotecan, level 1 was 120 mg/m^2 and level 2 was 100 mg/m^2; no increase was made once dosage decreased. Also, in the mFOLFIRI + bevacizumab regimen, dosage was reduced in patients with grade ≥4 leucopenia or neutropenia, grade ≥3 thrombocytopenia, diarrhoea, stomatitis, nausea or vomiting or non-haematological toxicity as follows: 120 mg/m^2 of irinotecan and 200 mg/m^2 of 5-FU (bolus) for level 1, and 100 mg/m^2 of irinotecan, 200 mg/m^2 of 5-FU (bolus) and 2,000 mg/m^2 of 5-FU (infusion) for level 2.

With regard to safety data, the patients' health status was observed and blood samples were tested during weekly medical examinations by the attending physician until 4 weeks after commencing treatment and repeated after the fifth week at the start of each new course of treatment. Adverse events were evaluated according to the Common Terminology Criteria for Adverse Events version 3.0, and effectiveness was observed according to the Response Evaluation Criteria in Solid Tumors 1.0. Computed tomographic scans were performed every 6 weeks. Effectiveness was judged comprehensively using blinded tests on the treatment methods by 3 or more physicians not including primary physicians.

Interim Analysis about Safety

After 3 cases have been registered in each group, registration was stopped to evaluate the safety of the two treatments (step 1). After the confirmation of the safety of the two treatments by the efficacy and safety evaluation committee, registration was reopened with 60 patients enrolled (30 per group; step 2).

Statistical Analysis

While attempting to detect a frequency of ≥10% with 95% probability for the occurrence of adverse events, we determined that the sample size would include 30 patients in each experimental group or 60 patients overall in the two experimental groups [19]. Patients' background, safety and efficacy data were summarized as frequencies and percentages. The χ^2 test was used to compare between groups, while the Kaplan-Meier method was used to analyse PFS.

Results

Patient Background

From November 2007 to February 2010, 60 patients were registered from the 12 institutes of the Tohoku Clinical Oncology Research and Education Society. These patients were randomly assigned to either the mFOLFIRI + bevacizumab or sequential IRIS + bevacizumab groups, with 30 patients in each group. Patient backgrounds are presented in table 1; the median age was 62.5 (range 46–77) and 62 years (range 31–73) in the mFOLFIRI + bevacizumab and sequential IRIS + bevacizumab group, respectively. Many patients were receiving first-line treatment (24 patients in the mFOLFIRI + bevacizumab group and 23 patients in the IRIS + bevacizumab group). No significant bias was seen between the two groups.

Safety Verification Test (Step 1)

Step 1 of this trial was to register 3 patients at a time into the two experimental chemotherapy regimen groups and evaluate the initial safety for 12 weeks. The last patient was registered in April 2008 when patient registration was temporarily suspended and initial safety was assessed. Except for 1 patient in the mFOLFIRI + bevacizumab group with gastrointestinal perforation (G3), no other severe adverse events occurred. Because international phase III and verification trials in combination with FOLFOX treatment in a Japanese population cite gastrointestinal perforation as an expected adverse event, the efficacy and safety evaluation committee recommended proceeding to step 2 while maintaining utmost vigilance with regard to patient safety.

Safety Verification Trial (Step 2)

By February 2010, 60 patients had been registered in the study, including the 6 patients from step 1 and were randomly allocated to the two experimental groups (table 1). Although one adverse event of gastrointestinal perforation (G5) was observed in the mFOLFIRI + bevacizumab group, this was determined to be due to progression of an underlying disease (table 2) and not due to the experimental treatment. With regard to G3/4 haematological toxicities in the mFOLFIRI + bevacizumab and sequential IRIS + bevacizumab treatment groups, neutropenia was seen at a rate of 48 and 38%, respectively. Although statistical differences were not observed, G3/4 gastrointestinal toxicities were more frequent in the mFOLFIRI + bevacizumab group than in the sequential IRIS + bevacizumab group (anorexia 17.9 and 3.4%, nausea 7.1 and 0%, diarrhoea 14.3 and 6.9%, respectively). G3/4 severity in hypertension, which is the representative adverse event of bevacizumab, was confirmed as 3.6% in the mFOLFIRI + bevacizumab group, whereas it was not observed in the sequential IRIS + bevacizumab group. No patient experienced severe proteinurea, thrombosis or haemorrhage in either group.

Comparison of Efficacy

The treatment methods were blind, and efficacy was compared by judging the response rate with a 3-person decision committee. The overall response rate (ORR) in the mFOLFIRI + bevacizumab group versus the sequential IRIS + bevacizumab group was 61.5% [95% confidence interval (CI) 40–80] and 72.0% (95% CI 51–86), respectively (table 3). Two patients showed complete response in the sequential IRIS + bevacizumab group. The median PFS was 324 days (95% CI 247–475) in the mFOLFIRI + bevacizumab group and 345 days (95% CI 312–594) in the sequential IRIS + bevacizumab group (fig. 1). Statistical significance was not observed between the two groups (p = 0.71).

Table 1. Characteristics of patients

	mFOLFIRI + bevacizumab (n = 30)	IRIS + bevacizumab (n = 30)
Age, years		
Median	62.5	62
Range	46–77	31–73
Males/females	18/12	17/13
ECOG performance status		
0	24	27
1	6	3
Primary legion		
Colon	17	17
Rectum	12	13
Both	1	0
Cancer		
Advanced	22	20
Recurrent	8	10
Histology		
Well	7	7
Moderately	20	22
Poor	2	0
Other	1	1
Primary site		
Yes	5	6
No	25	24
Number of metastases		
1	17	16
2	9	10
3	4	4
Adjuvant chemotherapy		
Yes	5	7
No	25	23
Prior chemotherapy		
Yes	24	25
No	6	5

ECOG = Eastern Cooperative Oncology Group.

Discussion

Systemic chemotherapy against unresectable or recurrent colorectal cancer was developed on the basis of the successful combination therapy of 5-FU and L-leucovorin. Continuous 5-FU infusion and cytotoxic drugs (e.g. irinotecan and L-OHP, as well as other molecular target-

Table 2. Adverse events of the two treatments

Adverse event	mFOLFIRI + bevacizumab							IRIS + bevacizumab							p value (χ^2 test; G3,4)
	G0	G1	G2	G3	G4	G5	grade >3, %	G0	G1	G2	G3	G4	G5	grade >3, %	
Non-haematological															
Anorexia	10	5	8	5			17.9	13	10	5	1			3.4	0.076
Nausea	10	7	9	2			7.1	16	11	2				0.0	0.143
Vomiting	20	6	1	1			3.6	28			1			3.4	0.980
Diarrhoea	12	12		4			14.3	15	11	1	2			6.9	0.364
Mucositis	17	10	1				0.0	23	6					0.0	(-)
Fatigue	14	8	4	2			7.1	17	9	3				0.0	0.143
GI perforation	26			1		1	7.1	29						0.0	0.143
Bleeding	20	7	1				0.0	21	8					0.0	(-)
Hypertension	20	3	2	1			3.6	24	2	1				0.0	0.304
Proteinuria	20	3	2				0.0	22	2	3				0.0	(-)
Haematological															
Leucopenia	5	6	12	4			14.3	12	3	9	5			17.2	0.409
Neutropenia	3[1]		11	8	5		48.1	12[1]		6	7	4		37.9	0.598
Thrombopenia	23	4					0.0	22	6		1			3.4	0.286

GI = Gastrointestinal. [1] Frequency of G0 and G1.

Table 3. Overall response of the two treatments

	mFOLFIRI + bevacizumab	IRIS + bevacizumab
CR	0	2
PR	16	16
SD	8	5
PD	2	2
NE	4	5
Total	30	30
RR, %	61.5 (40.1–79.8)	72.0 (CI 50.6–86.2)

Figures in parentheses are 95% CIs.
CR = Complete response; PR = partial response; SD = stable disease; PD = progressive disease; NE = not evaluated.

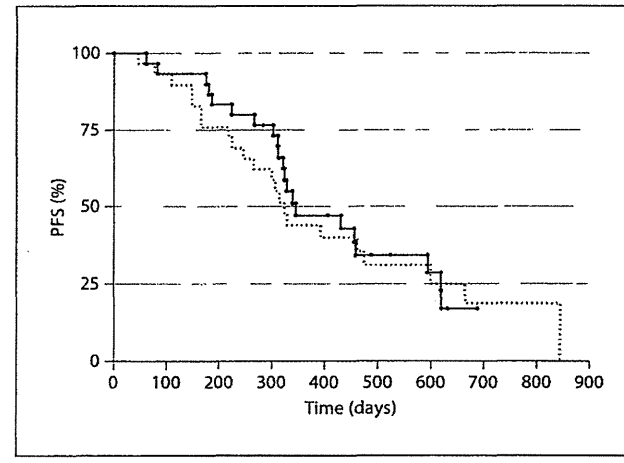

Fig. 1. Kaplan-Meier PFS curves of patients with metastatic colorectal cancer treated with mFOLFIRI + bevacizumab (dotted line) and IRIS + bevacizumab (solid line).

ed drugs, such as bevacizumab, cetuximab and panitumumab) are used concomitantly or sequentially to yield a median survival time that exceeds 2 years; however, continuous 5-FU infusion necessitates the insertion of a peripherally inserted central catheter or CV port, which can increase infection and thromboembolism risks. In order to circumvent these drawbacks, novel treatment options with oral fluoropyrimidines are being developed to replace the need for 5-FU infusions. The oral fluoropyrimidine S-1 exhibits a lower frequency of diarrhoea and hand-foot syndrome when compared with capecitabine, and S-1 has a higher tolerance level among Japanese people. Therefore, treatments such as SOX and IRIS are being developed in Japan to replace FOLFOX and FOLFIRI therapies, and it has been suggested that S-1 may be

able to replace 5-FU/LV [12–14]. Furthermore, because molecular targeted drugs, such as bevacizumab, cetuximab and panitumumab, have been introduced into routine clinical use in Japan, it has become important to evaluate the safety and efficacy of combined therapies on the basis of these drugs and on the new oral fluoropyrimidines.

Prior to this study, we tested the safety and efficacy of sequential IRIS therapy, which we found to have a low toxicity and high efficacy [13]. In this study, among patients with G3 or higher haematological toxicities, no significant differences between the two groups were observed with regard to neutropenia and/or leucopenia, although a lower trend was observed in the sequential IRIS + bevacizumab group. Muro et al. [16] performed a phase II/III trial comparing mFOLFIRI with irinotecan + S-1 therapy as a second line of treatment for patients with unresectable recurrent colorectal cancer. Although their administration method differed from our sequential IRIS therapy, as Muro et al. [16] did not use bevacizumab in their study, the frequency of G3/4 neutropenia in the mFOLFIRI (150 mg/m^2/2 weeks of irinotecan) and IRIS groups showed a similar trend to our data (52.1 and 36.2%, respectively), indicating that IRIS exhibits less neutropenic toxicity.

The incidence of gastrointestinal toxicity observed in this study in the mFOLFIRI + bevacizumab group was nearly identical to that in the FOLFIRI group (43.2–53.6%) as reported by a BICC-C study [4]. As with haematological toxicities, the frequency of non-haematological toxicity was lower in the sequential IRIS + bevacizumab group than in the mFOLFIRI + bevacizumab group. Furthermore, the frequency of reported gastrointestinal toxicities, such as loss of appetite (11%) and diarrhoea (20.5%), in the sequential IRIS + bevacizumab group of our study tended to be lower than that in the IRIS group in the study of Muro et al. [16]. This difference may be due to the following reasons: (1) all patients in the study of Muro et al. [16] were undergoing second-line treatment, and (2) the different administration method used placed a greater emphasis on irinotecan dose intensity than our sequential IRIS method. Muro et al. [16] also mentioned that raising the dose intensity of irinotecan was among the effective strategies for patients resistant to oxaliplatin-based chemotherapy; however, with regard to these adverse events, we believe that raising the dose intensity of S-1 rather than that of irinotecan is the better strategy for first-line treatment with regard to safety. Finally, as regards efficacy, the median PFS in both groups was about nearly a year. Although the number of patients in the current study was small, the level of efficacy seems to be higher than that in previous studies. The data on overall survival time are currently being analysed in a follow-up study.

Recently, Yamada et al. [20] reported the results of a phase II study on IRIS combined with bevacizumab (SIRB study). In the SIRB regimen, S-1 is administered on days 1–14 of a 21-day cycle, but the dose intensity of S-1, irinotecan and bevacizumab was equivalent to that of the sequential IRIS + bevacizumab regimen. Toxicity in the SIRB regimen was low and manageable (G3/4 neutropenia 26%, G3/4 anorexia 12%, G3/4 diarrhoea 8%). The ORR was 67% (95% CI 52.1–79.1) and the median PFS was 373 days (95% CI 299–440), which is comparable with our sequential IRIS + bevacizumab therapy.

From these results, we concluded that the combination of S-1, irinotecan and bevacizumab could be an effective primary therapy in Japanese patients, compared with mFOLFIRI + bevacizumab. Moreover, this regimen could reduce the risk of infection because it does not require a CV port. Therefore, sequential IRIS + bevacizumab therapy, a very promising treatment method, should be developed further in a larger randomized clinical trial. We are currently in the process of planning a phase III clinical trial in Japan comparing IRIS + bevacizumab with CapOX/FOLFOX + bevacizumab.

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ORIGINAL ARTICLE

Influence of a multidisciplinary cancer board on treatment decisions

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Abstract
Background To clarify how a multidisciplinary cancer board (CB) influences treatment decisions.
Methods From March 2010 to June 2011, a total of 475 cases were discussed at our CB and the minutes of the board were reviewed for this study.
Results Of the 475 patients, minor changes in treatment methods were made in 42 patients (9 %) and major changes were made in 28 patients (6 %). Further diagnostic procedures, further publication surveys and reconfirmation of patient's wishes were recommended in 80 patients (17 %). In the 392 patients for whom treatment was recommended, the CB's recommendation was realized in 349 patients (89 %) and was not realized in 20 (5 %) patients.
Conclusions It is obvious that a CB has a great influence on cancer treatment decisions, but the effectiveness of the CB in our hospital should be verified in the future by analyzing treatment outcomes.

Keywords Cancer board · Multidisciplinary approach · Treatment decision

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Introduction

As medical practice becomes increasingly specialized, a more comprehensive and multidisciplinary approach is being utilized to diagnose and treat various kinds of cancer. In recent years, conferences in which various specialists including physicians, surgeons, radiation oncologists, medical oncologists, radiologists, pathologists, and palliative care specialists meet to discuss diagnosis and treatment have been become popular in western countries [1]. This kind of meeting is called a cancer board (CB).

Cancer boards are also becoming popular in Japan, especially in designated cancer hospitals. In September 2008, we established a multidisciplinary CB in Yamagata University Hospital to determine best treatment recommendations, and about 400 cases have been discussed every year. However, the impact of the CB on treatment decisions has not been investigated in detail. In this study, we analyzed the results of discussions and investigated how the CB has influenced treatment decisions.

Materials and methods

Yamagata University Hospital

Yamagata Prefecture has a population of 1.2 million and the Japanese government has designated 6 cancer hospitals in the prefecture. Yamagata University Hospital is a general hospital with 17 clinical departments and 625 beds and is one of the regional designated cancer hospitals. In 2010, 1337 new cancer patients were treated at the hospital.

Table 1 Timetable of the cancer board in Yamagata University Hospital

Time	17:00	17:30	18:00	18:30	19:00
1st week					
Tuesday	Lung	Bone/soft tissue	Gastrointestinal/hepatobiliary	Brain	
Wednesday	Hematology/pediatric	Head and neck	Other		
2nd week					
Tuesday	Lung	Gynecology	Gastrointestinal/hepatobiliary	Urology	
3rd week					
Tuesday	Lung	Bone/soft tissue	Gastrointestinal/hepatobiliary	Brain	Breast
Wednesday	Hematology/pediatric	Head and neck	Other		
4th week					
Tuesday	Lung	Gynecology	Gastrointestinal/hepatobiliary	Urology	

CB in Yamagata University Hospital

In our hospital, CBs are held every Tuesday and on alternate Wednesdays. The timetable of CBs is shown in Table 1. In the evening of each of those days, a meeting of 13 boards is held in the same room with various types of equipment for presenting data from an electronic medical record system and images from a radiology information system. Because of restrictions in manpower, discussion time for each board is usually less than 30 min. Cases are presented after diagnosis has been made, and the discussion is focused on the best treatment for each case. Attendees include physicians, nurses, pharmacists, and medical students. To promote a multidisciplinary approach, at least one medical oncologist and radiation oncologist (usually two or more) have participated in the CBs. A palliative care specialist has also participated in most of the CBs.

Realization of CB recommendations

To investigate the realization of CB recommendations, clinical records were reviewed to determine whether the recommended treatment was given for each patient.

Results

From March 2010 to June 2011, a total of 475 cases were discussed at CBs, and the minutes of the boards were reviewed for this study. The classification of the CB determinations is shown in Table 2. Minor changes include changes in chemotherapy drugs, dose of drugs, and dose of radiation. Major changes include change from surgery to chemoradiation and from palliative care to curative therapy. If more detailed examination or more detailed survey of publications is required, the board determination is classified as pending. The tumor status of cases discussed at the CBs is summarized in Table 3. Of 475 patients, only 216 patients (45 %) had a new tumor and more than half of the patients had a recurrent or residual tumor. Cases that were presented several times at CBs are counted as different cases.

Table 2 Classification of CB determinations

I	Approval of the proposed treatment
II	Selection of a treatment from several options
IIIA	Minor change (e.g., drug type, dose of radiation)
IIIB	Major change (e.g., palliative care to curative treatment)
IV	Pending (e.g., add examination, survey more publications)
V	Others

Table 3 Tumor status discussed at cancer board

Tumor status	Total
Untreated cases	216 (45 %)
Recurrent cases	179 (38 %)
Residual disease after initial therapy	67 (14 %)
Other	13 (3 %)
Total	475

The number of cases discussed at each board and influence of the CB on treatment decisions are shown in Table 4. In our institution, the largest number of cases was discussed at the hematology board followed by the lung board, urology board, and head and neck board. Breast cancer and hepatobiliary cancer are not rare in our hospital, but the number of cases discussed at the CB was very small.

The CB had a great impact on treatment methods. In a total of 475 patients, minor changes in treatment methods were made in 42 patients (9 %) and major changes were made in 28 patients (6 %). Further diagnostic procedures, further publication surveys, and reconfirmation of the patient's wishes were recommended in 80 patients (17 %).

Table 4 Number of cases discussed at each board and influence of the CB on treatment determination

Board	I	II	IIIA	IIIB	IV	V	Total
Brain	21 (41 %)	10 (20 %)	5 (10 %)	3 (6 %)	12 (24 %)	–	51 (100 %)
Head and neck	21 (36 %)	21 (36 %)	7 (12 %)	2 (3 %)	7 (12 %)	–	58 (100 %)
Lung	14 (20 %)	26 (38 %)	10 (14 %)	2 (3 %)	17 (25 %)	–	69 (100 %)
Breast	2 (67 %)	1 (33 %)	–	–	–	–	3 (100 %)
Gastrointestinal	12 (23 %)	22 (42 %)	4 (8 %)	5 (9 %)	10 (19 %)	–	53 (100 %)
Hepatobiliary	2 (50 %)	1 (25 %)	1 (25 %)	–	–	–	4 (100 %)
Urology	33 (48 %)	17 (25 %)	6 (9 %)	3 (4 %)	10 (14 %)	–	69 (100 %)
Gynecology	8 (19 %)	15 (36 %)	3 (7 %)	8 (19 %)	8 (19 %)	–	42 (100 %)
Bone and soft tissue	8 (42 %)	5 (26 %)	1 (5 %)	5 (26 %)	–	–	19 (100 %)
Pediatric	2 (29 %)	4 (57 %)	–	–	1 (14 %)	–	7 (100 %)
Ophthalmology	1 (25 %)	–	–	–	2 (50 %)	1 (25 %)	4 (100 %)
Hematology	56 (65 %)	13 (15 %)	5 (6 %)	–	10 (12 %)	2 (2 %)	86 (100 %)
Other	–	7 (70 %)	–	–	3 (30 %)	–	10 (100 %)
Total	180 (38 %)	142 (30 %)	42 (9 %)	28 (6 %)	80 (17 %)	3 (1 %)	475 (100 %)

Of 28 group IIIB patients, best supportive care instead of curative therapy was recommended in 5 patients. Definite therapy instead of best supportive care was recommended in 7 patients (surgery in 3 patients, chemoradiotherapy in 3 patients, and radiation therapy alone in one patient). For the other 16 patients, recommended treatments instead of scheduled treatment were particle radiotherapy (carbon ion or proton) in 6 cases, surgery in 3 cases, chemotherapy in 2 cases, chemoradiotherapy in 2 cases, and other therapy in 3 cases.

Of the 392 patients in whom treatment was recommended, the CB's recommendation was realized in 349 patients (89 %) and was not realized in 20 patients (5 %). The main reason for the CB's recommendation not being realized was the patient's refusal of the proposed treatment [17 (85 %) of the 20 patients]. In 23 patients, realization of the CB's recommendation could not be followed.

Discussion

As shown in Table 4, there is a wide range in the case numbers discussed at the CB. In our institution, presentation of cases at the CB is recommended, but not all of the cases treated in our institution are presented at the CB. Our CB schedule is not sufficient to discuss all cancer cases because each board discussion is limited to 30 min. The reason for the differences in activities of the boards is not clear, but the motivation of physicians seems to be different depending on the departments and it appears that some physicians think that discussion of treatment for each patient at the CB is not necessary. Another reason is the ratio of patients for whom standard treatments can be applied. If standard treatments can be applied for a large proportion of specific cancer patients, physicians may think it is unnecessary to present the cases at the CB.

More than half of the cases discussed at the CB had a recurrent or residual tumor. In our hospital, more than one thousand new cancer patients are treated every year, and the number of cases presented at the CB is only a proportion of the cases. As mentioned above, many patients for whom standard treatment methods can be applied may have been treated without CB presentation. In contrast, for many recurrent or residual cancer cases there is no standard treatment and many of them may have been presented at the CB for consultation.

Changes in treatment methods were recommended by many CBs. Minor and major changes in treatment were recommended in 9 and 6 % of the patients, respectively, and a treatment decision was not made in 17 % of the patients. Wheless et al. [2] reported that in the head and neck board, treatment change was recommended in 24 % of the patients and that more patients received more intensive therapy. Kurpad et al. [3] reported that in the urologic board, changes in treatment were most common in bladder cancer (44 %), followed by kidney (36 %), testicular (29 %), and then prostate (22 %) cancers. The ratio of patients whose treatment was changed is low in our series. We discuss treatment recommendation mainly for patients after full diagnostic procedures, because discussion time for each board is limited to 30 min. We therefore do not have enough time for discussions about diagnosis. However, it has been reported that changes in pathologic diagnosis and radiologic diagnosis are frequent at a CB [4–6]. Gatcliffe [5] reported that changes were recommended in 53 of 153 presented cases. Major changes ($n = 13$) predominantly resulted from pathology reassignments. Minor changes ($n = 40$) resulted from pathology, staging,

radiology, and surgical team clarifications. Changes in diagnosis should influence treatment, but changes in diagnosis were rare in our CB and this may be a reason for the low ratio of patients whose treatment was changed.

Sarff et al. [7] reported that 42 % of the participants in their study indicated that CB information would change their practice. In our hospital, many residents and medical students participate in various CBs and the CB is a good chance for them to improve their knowledge of oncology.

Shortage of medical and radiation oncologists is a great problem in Japan, and there are many hospitals, including designated cancer hospitals, without oncologists. In hospitals with oncologists, the number of staff is very small and it is difficult to attend many kinds of CBs. In such hospitals, hiring part-time oncologists for the CB may be useful. In fact, in our area, attendance of part-time oncologists at CBs is becoming common.

It is obvious that a CB has a great influence on cancer treatment decisions, but the main goal of a CB is to improve treatment outcomes such as survival and quality of life. However, the effect of a CB on treatment outcomes has been investigated in only a few studies. In a retrospective study, median survival time of patients with advanced lung cancer was shown to have been prolonged by the CB from 3.2 to 6.6 months [8]. A possible reason for this improvement was that the CB decreased the use of palliative care only and increased the use of chemotherapy. Junor et al. [9] analyzed prognostic factors in ovarian cancer patients and found that treatment in a joint clinic (multidisciplinary team) was a prognostic factor. The effectiveness of the CB in our hospital should be verified in the future by analyzing treatment outcomes.

Conflict of interest The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Phase I study of irinotecan by 24-h intravenous infusion in combination with 5-fluorouracil in metastatic colorectal cancer

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Abstract
Background This study was intended to ascertain the feasibility of a combination therapy with irinotecan by 24-h intravenous infusion (24-h CPT-11) and 5-fluorouracil (5-FU) for patients with metastatic colorectal cancer, to estimate the dose-limiting toxicity (DLT) and the maximum tolerated dose (MTD), to determine the recommended dose (RD) for the Phase II study, and to evaluate the efficacy of the combination therapy.
Methods The dosage regimen was as follows: CPT-11 was given by 24-h CPT-11 on day 1, followed by 24-h intravenous infusion of 5-FU on day 2. This regimen was repeated every 2 weeks. The dose of CPT-11 was escalated in five steps from 50 to 75, 100, 125, or 150 mg/m^2 (levels 1–5), whereas the dose of 5-FU was fixed at 800 mg/m^2.
Results Twenty-six patients were recruited for this study, and 25 of the 26 patients were eligible for the assessment. The DLTs of 24-h CPT-11/5-FU therapy included grade 3 diarrhea in 1 patient treated at level 1, and grade 3 neutropenia in 1 patient and grade 4 neutropenia in 1 patient at level 4. In level 5, in 3 cases the next administration could not be done for 22 days or more as a consequence of anorexia. Thus, the level 5 was made a MTD and the level 4 was made a RD. The main side effects of grade 3 or higher, although nausea/vomiting occurred, were mild and tolerable in severity overall. The overall response rate was 24.0% (6PR/25).
Conclusion This study suggests that 24-h CPT-11/5-FU therapy is feasible and effective for treatment of metastatic colorectal cancer.

Keywords Colorectal cancer · Irinotecan (CPT-11) · 5-Fluorouracil (5-FU)

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Introduction

5-Fluorouracil (5-FU), which was introduced in 1958, has kept its position as a key drug for chemotherapy of colorectal cancer for about 40 years. 5-FU alone has been investigated for the dosage regimen by bolus injection, intravenous infusion, or other methods of administration primarily in the United States and Europe. In the late 1980s, combination chemotherapy with leucovorin (LV) was studied based on the biochemical modulation theory. This combination chemotherapy has been established as a standard treatment of colorectal cancer. Irinotecan (CPT-11) is

a camptothecin derivative extracted from *Camptotheca acuminata*. It has been recognized that CPT-11 exerts potent tumor-reducing activity by inhibiting DNA topoisomerase I (topo-I) [1]. A synergetic effect is observed between CPT-11 and 5-FU when they are administrated sequentially, and CPT-11 followed by 5-FU shows a better effect [2]. In addition, an attempt has been made to use irinotecan by weekly 24-h infusion as the second-line therapy for metastatic colorectal cancer, and the usefulness of this regimen has been suggested [3]. Especially, a Phase III study conducted mainly in the United States and Europe demonstrated that CPT-11/5-FU/LV combination therapy results in a survival benefit in patients with colorectal cancer. Currently, CPT-11/5-FU/LV has been established as the standard first-line therapy for colorectal cancer [4, 5].

A preclinical study suggested that a higher antitumor activity of CPT-11 is produced by long-term exposure with continuous intravenous infusion at a low dose to tumors than by exposure by short infusion with high dose intensity because the activity of CPT-11 is schedule dependent, although not markedly so [6]. Thus, a new approach by 24-h intravenous infusion of CPT-11 has been investigated for treatment of colorectal cancer [3, 7, 8].

We conducted a Phase I study to ascertain the feasibility of a combination therapy with CPT-11 by 24-h intravenous infusion and 5-FU for patients with metastatic colorectal cancer, to estimate the dose-limiting toxicity (DLT) and the maximum tolerated dose (MTD), to determine the recommended dose for the Phase II study, and to evaluate the efficacy of this combination therapy.

Patients and method

Patient eligibility

Inclusion criteria were as follows: (1) patients with histologically proven colorectal cancer; (2) patients with measurable or assessable lesions; (3) patients whose major organ functions were maintained adequately (white blood cells $\geq$ 4,000/mm^3; neutrophils $\geq$ 2,000/mm^3; platelets $\geq$ 100,000/mm^3; hemoglobin $\geq$ 9.5 g/dl; AST/ALT $\leq$ 2.5 $\times$ institutional upper limit of normal AST/ALT; total serum bilirubin $\leq$ 2.0 mg/dl; BUN $\leq$ 25 mg/dl; serum creatinine $\leq$ 1.5 mg/dl; creatinine clearance $\geq$ 50 ml/min; and normal ECG, excluding cardiac arrhythmias and ischemic changes); (4) patients whose performance status (ECOG) was 0–2; (5) patients who were free from carryover effects or adverse reactions from prior treatment; (6) life expectancy $\geq$ 3 months; (7) age $\geq$ 15 years and $\leq$ 75 years; and (8) patients who gave written informed consent.

Exclusion criteria were as follows: (1) severe fluid retention (pleural effusion or ascites); (2) metastasis to the central nervous system (CNS); (3) fresh bleeding from gastrointestinal tract; (4) diarrhea (watery stool); (5) infections; (6) intestinal paralysis or intestinal obstruction; (7) interstitial pneumonia or pulmonary fibrosis; (8) uncontrolled diabetes; (9) cardiac failure, renal failure, or hepatic failure; (10) active double cancer; (11) active psychiatric disorder; (12) previous abdominal irradiation; (13) pregnant women, nursing mothers, or women of childbearing potential; and (14) any patients who were judged to be inappropriate for the study by the investigator.

Treatment and dose escalation schedule

CPT-11 was administered by 24-h intravenous infusion on day 1, followed by 24-h intravenous infusion of 5-FU on day 2 every 2 weeks. For the dose-finding study, the dose levels were determined for three patients at each level, as a rule, a modified Fibonacci scheme [9]. Although the dose of 5-FU was fixed at 800 mg/m^2, dose levels of CPT-11 were escalated in five steps (levels 1–5) from 50 mg/m^2 as the starting dose to 75, 100, 125, and 150 mg/m^2. Each dose level was assessed for DLTs developing until the second course of treatment. Based on the assessment of DLT developing at the dose level, it was determined whether inclusion of additional patients and escalation to the next level were acceptable.

Dose-limiting toxicity (DLT) and maximum tolerated dose (MTD)

Dose-limiting toxicity (DLT) was defined as follows: (1) grade 3 or 4 hematological toxicity, (2) grade 3 or 4 leukopenia or neutropenia accompanied with a fever >38.0°C, (3) grade 3 or 4 nonhematological toxicity (excluding nausea/vomiting, anorexia, and alopecia), and (4) an event such that the next infusion was not carried out within 22 days after the previous infusion.

To determine the maximum tolerated dose (MTD), three patients were enrolled at each level. If none of the three patients developed any DLT, the dose of CPT-11 was escalated to the next level. If one or two of three patients developed a DLT, then three additional patients were enrolled at the same dose level. If three of six patients developed a DLT, the current level was considered as the MTD. If not more than two of the six patients developed a DLT, the dose of CPT-11 was escalated to the next level. If all three patients developed a DLT, the current level was considered as the MTD.

Assessment

Adverse reactions were evaluated according to the WHO Common Toxicity Criteria. The antitumor effect was

evaluated according to the Efficacy Evaluation Criteria in Solid Cancer of the Japan Society of Clinical Oncology.

Pharmacokinetics

Plasma concentrations of CPT-11 and its metabolite SN-38 during combination therapy with 24-h CPT-11 and 5-FU were examined. Blood samples were collected at the following time points: 1, 6, 12, 24 (equal to end of CPT-11 infusion), 25, 27, 30, 36, and 48 h after start of CPT-11 infusion. The volume of blood collected was 2 ml each, and at least 1 ml plasma was collected by centrifuge. The analytes were determined by high-performance liquid chromatography.

Results

Patient population

Twenty-six patients were recruited for this study, and 25 of the 26 patients were eligible for the assessment, excluding 1 patient who had diarrhea before the start of infusion. The demographic and baseline characteristics of the 25 patients are shown in Table 1.

Dose-limiting toxicity and other toxicities

Major adverse reactions reported during the study are shown in Table 2. DLTs included grade 3 diarrhea in one patient at level 1, grade 3 neutropenia in one patient at level 4, grade 3 leukopenia and grade 4 neutropenia in one patient at level 4. In level 5 (CPT-11 150 mg/m^2), in three cases the next administration could not be done for 22 days or more as a consequence of anorexia. In addition, hematological toxicities including grade 1–2 anemia in seven patients were observed. Nonhematological toxicities included nausea/vomiting. Generally, all toxicities were mild or moderate and tolerable.

Maximum tolerated dose and recommended dose

In this study, with level 5 (CPT-11 150 mg/m^2, 5-FU 800 mg/m^2), because there were three of six cases in which the next administration was delayed for 22 days or more because of toxicity, this level was made the MTD. As a result, level 4 (CPT-11 125 mg/m^2, 5-FU 800 mg/m^2) was made the recommended dose (RD) of 24 h CPT-11/5-FU therapy.

Antitumor activity

The antitumor effect was not used as the primary endpoint. The antitumor effect in 25 evaluable patients was 6 partial response (PRs), 9 no change (NCs), and 10 progressive disease (PDs): the response rate was 24.0% (95% CI, 7.3–40.7%) (colon cancer, 16.7%; rectal cancer, 30.8%). According to dose levels, 3 PRs, 1 NC, and 2 PDs in 6 patients occurred at the recommended dose, level 4: the response rate was 50.0% (95% CI, 10.0–90.0%].

The median time to response was 28 days (range, 7–74 days), and the duration of response (median) was 90 days (range, 48–165 days).

Pharmacokinetics

Changes in the plasma concentration of CPT-11 showed almost the same pattern at all levels. The plasma concentration increased until 12–24 h after the start of infusion. After the completion of infusion, it decreased quickly, and reached approximately the quantitation limit 24 h after the completion of infusion. As the dose of CPT-11 at each

Table 1 Patient characteristics

	Level 1	Level 2	Level 3	Level 4	Level 5	Total
No. of patients	6	4	3	6	6	25
Gender						
Male/female	4/2	4/0	3/0	3/3	5/1	19/6
Age						
Median (range)	62 (57–70)	61 (55–61)	56 (55–61)	51 (36–60)	53 (43–65)	58 (34–70)
PS (ECOG) 0/1/2	1/3/2	1/3/0	1/2/0	3/2/1	2/4/0	8/14/3
Primary colon/rectum	3/3	3/1	2/1	3/3	1/5	12/13
Metastatic site						
Liver	1	1	2	0	3	8
Lung	4	3	1	3	4	15
Lymph nods	3	0	0	4	1	8
Others	1	1	0	1	0	3

Table 2 Toxicity

Level (CPT-11 dose)	No. of patients	Leukopenia			Neutropenia			Anemia			Diarrhea			Nausea/vomiting			Anorexia		
		Grade			Grade			Grade			Grade			Grade			Grade		
		3	4	≥Gr 3 (%)	3	4	≥Gr 3 (%)	3	4	≥Gr 3 (%)	3	4	≥Gr 3 (%)	3	4	≥Gr 3 (%)	3	4	≥Gr 3 (%)
1 (50 mg/m^2)	6	0	0	0	0	0	0	0	0	0	1	0	16.7	0	0	0	1	0	0
2 (75 mg/m^2)	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3 (100 mg/m^2)	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4 (125 mg/m^2)	6	1	0	16.7	1	1	33.3	0	0	0	0	0	0	1	0	16.7	1	0	0
5 (150 mg/m^2)	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0

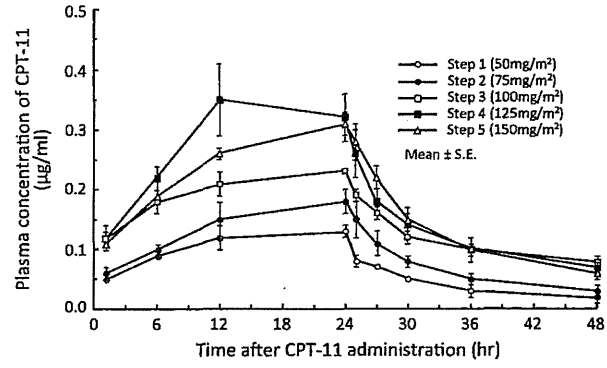

Fig. 1 Mean plasma concentrations of irinotecan (CPT-11) after drip infusion of CPT-11 and 5-fluorouracil (5-FU) (800 mg/m^2) in humans

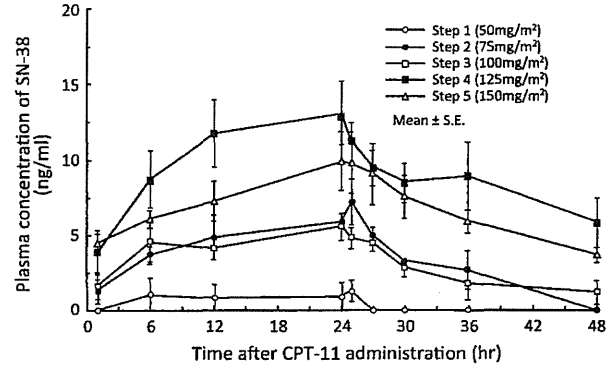

Fig. 2 Mean plasma concentrations of SN-38 after drip infusion of CPT-11 and 5-FU (800 mg/m^2) in humans

level increased, the plasma concentration increased (Fig. 1). The concentration of SN-38 reached a peak 24–25 h after the start of infusion. However, a consistent pattern of changes in plasma concentrations of SN-38 was not observed among levels, and both increase and decrease in the plasma concentration occurred more slowly than those of CPT-11. No dose-dependent pattern was observed for the plasma concentration of SN-38 (Fig. 2).

Discussion

We conducted a Phase I study of combination therapy with CPT-11 by 24-h intravenous infusion with 5-FU at the Institute of Development, Aging and Cancer, Tohoku University and two other institutions. The study confirmed that this therapy was feasible for the treatment of patients with metastatic colorectal cancer. Major adverse reactions were grade 3 diarrhea, grade 3/4 neutropenia, and delayed administration for more than 22 days because of adverse reactions; these were dose-limiting toxicities (DLTs). Most

other adverse reactions were mild or moderate and well tolerable. The doses of 24-h CPT-11/5-FU therapy up to level 5 were below the MTD. Level 4 (CPT-11 125 mg/m^2 on day 1 and 5-FU 800 mg/m^2 on day 2) was regarded as the RD.

In the analysis for overall response, six patients achieved PR with a response rate of 24%. Among the other patients, ten had NC and none had PD. Among six patients in level 4, which is the RD, three achieved PR with a response rate of 50%; of the others, one had NC and two had PD.

Nowadays, the regimen adding a molecular targeted agent such as bevacizumab and cetuximab to infusional 5-FU/LV/CPT-11 (FOLFIRI) and infusional FU/LV/L-OHP (FOLFOX) is widely used as the standard therapy in metastatic colorectal cancer [10, 11]. Especially, CPT-11 is recommended for the second treatment or later. In that case, several administration methods that alleviate adverse reactions are necessary in consideration of the impact from previous treatments.

Furthermore, our study was designed on the assumption that 24-h intravenous infusion would be an appropriate dosing method based on its drug profile because CPT-11 has a schedule-dependent mechanism of action, although not markedly so.

The recommended dose of CPT-11 with 5-FU at a fixed dose of 800 mg/m^2 was determined by reference to the schedule in JCOG9703 in which LV was not included [12]. As a result, this 24-h CPT-11/5-FU therapy showed a better effect with lower incidence of adverse events than FOLFIRI, previously reported as the second-line treatment [13, 14].

Mild toxicity in this 24-h CPT-11/5-FU therapy is similar to that reported by other studies which examined 24-h CPT-11 with UFT or UFT/LV [7, 8].

In the analysis of drug disposition, the CPT-11 to SN-38 conversion seems to decrease. Our study suggested that 24-h CPT-11/5-FU therapy is effective for treatment of metastatic colorectal cancer because the high safety of the therapy was demonstrated in patients with metastatic colorectal cancer, although grade 3 or 4 hematological toxicities, which could be resolved by supportive treatment, were seen, and the response rate was 50% at the recommended dose (level 4). In addition, a biweekly treatment schedule is suitable for ambulatory chemotherapy. A biweekly treatment schedule might be useful to complete the treatment program because the drug-free period of about 2 weeks would allow recovery from adverse reactions occurring during the treatment.

In conclusion, 24-h CPT-11/5-FU combination therapy for metastatic colorectal cancer may be a worthy regimen to evaluate endpoints including progression-free survival and overall survival in a Phase II study.

Conflict of interest Y. Ohashi received lecture fees and manuscript fee from Daiichi Sankyo. The other authors have no conflict of interest.

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Practical Utility of Circulating Tumour Cells as Biomarkers in Cancer Chemotherapy for Advanced Colorectal Cancer

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Abstract. *Molecular-targeted therapies require the assessment of targets and their related molecules. Circulating tumour cells (CTCs) are considered a very good source of samples for these purposes. In this study, we applied a practical method for examining CTCs to evaluate the effects of chemotherapy on advanced colorectal cancer (CRC). Even in stage IV CRC, CTCs were detected in only 38.5% (n=5/13) of the cases. However, in cases where CTCs were detected, the change in the number of CTCs compared before and after chemotherapy appeared to be associated with the therapeutic outcome. Changes in the number of CTCs may be a good predictive biomarker. Problems with this method are yet to be resolved, including the detection rate and the stability of the sample source for subsequent molecular analysis.*

Recent advances in chemotherapy have been mainly due to the development of molecular-targeted agents. The use of these therapies depends on the molecular diagnosis related to the target molecules themselves or other molecules located in their signalling pathways. For the treatment of colorectal cancer (CRC), administration of antibodies to epidermal growth factor receptor (EGFR) is effective for patients with the wild-type *Kirsten rat sarcoma viral oncogene homolog* (*KRAS*) phenotype (1, 2). Genotyping of *v-Raf murine sarcoma viral oncogene homolog B1* (*BRAF*) and *phosphoinositide 3-kinase catalytic subunit* (*PI3CA*) should also be considered (1). In addition, overall expression profiling using products such as the 18-gene signature ColoPrint is under consideration for the molecular diagnosis of metastatic CRC (3). In any case, molecular diagnosis requires the use of DNA or RNA derived from resected specimens. Such samples are archival and thus do not represent the real-time status of the disease and its potential molecular targets. Furthermore, because almost all targets of chemotherapy for advanced-stage cancer are metastatic lesions, it is often difficult to obtain samples.

Analysis of circulating tumour cells (CTCs) from patients with cancer has recently become possible (4-6). CTCs are attractive sources for tumour analysis, as they can be obtained safely and are real-time tumour samples. The CellSearch system (Veridex LLC, Raritan, NL, USA), an immunomagnetic enrichment method, has been approved by the US Food and Drug Administration (7). In this method, ferrofluid coated with antibodies to epithelial cell adhesion molecule (EpCAM) is employed for the selection of epithelial cells. Antibodies to cytokeratin 8, 18, and 19 are also used for positive selection, and antibody to CD-45 is used for negative selection to eliminate leukocytes. Diamidino-2-phenylindole (DAPI), a marker of cell nuclei, is used in the negative selection of red blood cells and debris. In a present study, no healthy volunteer was found to have more than one CTC (4). CTC analyses have been included in several clinical trials (8, 9). Some of the results are promising, but further confirmation is needed.

In this study, we counted CTCs in blood from patient with stage IV CRC and analysed the clinical importance and utility of samples for molecular diagnosis. We demonstrate the potential usefulness of CTC analysis and note that further modification of the methodology is needed.

Patients and Methods

Fourteen patients with CRC stages IIIand IV treated at the Department of Clinical Oncology at Akita University Hospital from January 2012 to October 2012 were enrolled after acquiring their informed consent. This study was scientifically and ethically approved by the Committee of the School of Medicine of Akita University (#828).

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Table I. *Patient background.*

Case	Age (years)/Gender	Primary	Meta	Stage	CTC (n/7.5 ml)
1	76/F	Ce/tub1	(Li), Lym	IV	0, 0
2	68/M	A/tub1	Li, Lu	IV	2
3	60/F	T/tub1	Li	IV	4, 28, 73, 18, 12, 16, 6
4	65/M	R/tub1	Lu	IV	0, 0
5	57/F	A/tub2	Li	IV	1
6	78/F	Ce/tub1	Li	IV	0
7	77/M	A/tub1	PC	IV	0
8	68/F	R/tub1	(–)	III	0
9	52/M	R/tub2	Li	IV	0
10	66/M	R/tub1	Li, Lu	IV	0
11	80/M	R/tub1	Lu	IV	0, 0
12	68/M	T/tub2	Li, PC	IV	1
13	70/M	A/tub1	Lu, Li, PC	IV	1, 0
14	54/F	Ce/MAC	PC	IV	0

M, male; F, female; Ce, Cecum; A, ascending; T, transverse; R, rectum; Li, liver; Lym, lymph nodes; Lu, lung; PC, peritonitis carcinomatosa; tub1, well differentiated tubular adenocarcinoma; tub2, moderately differentiated; MAC, Mucinous adenocarcinoma.

Collection of CTCs. CTCs were obtained from 20 ml of peripheral venous blood drawn from each patient. CTCs were collected using the CellSearch kit (Veridex LLC, Raritan, NL, USA) and the Cell Tracks autoprep machine (Veridex LLC, Raritan, NL, USA). Identification of CTCs was confirmed using the Cell Tracks analyzer. In brief, CTCs were selected using anti-EpCAM and anti-cytokeratin antibodies (positive selection) and anti-CD-45 antibody (negative selection).

Mutation analysis of KRAS. DNA was extracted from CTCs and mutational analysis of *KRAS* was conducted using the Scorpion-ARMS real-time PCR method (10). The mutations analysed included Gly12Ala, Gly12Asp, Gly12Arg, Gly12Cys, Gly12Ser, Gly12Val, and Gly13Asp.

RNA extraction. RNA was extracted from CTCs using the NucleoSpin RNA XS kit (Takara Bio, Tokyo, Japan). CTCs are lysed by incubation in the lysis buffer. Residual genomic DNA is removed by on-column digestion with DNase, and total RNA was eluted.

Statistical analysis. The Pearson product-moment correlation coefficient between CTC number and therapeutic outcome was determined using STAT III mate (ATMS, Tokyo, Japan).

Results

Detection rate of CTCs in patients with stage IVCRC. Demographic information on the CRC cohort is presented in Table I. The age of the patients ranged from 52 to 80 years. Thirteen patients with stage IV CRC and one with stage III CRC were included. Primary sites of stage IV CRC were as follows: cecum (n=3), ascending colon (n=4), transverse colon (n=2), and rectum (n=5). Nine patients had liver metastases, five had lung metastasis, and four had cancerous peritonitis. The overall rate of CTC detection was 38.5% (n=5/13). In patients with liver metastases, the detection rate was particularly high (55.6%, 5/9), whereas CTCs were not detected in patients with stage IV CRC without liver metastasis. The number of CTCs was less than 2 cells per 7.5 ml of whole blood in 80% (4/5) of the CTC-positive cases. In only one case were CTCs detected repeatedly; the median number of CTCs was 16 per 7.5 ml of whole blood (range, 2–73). In cases 1, 4, and 11, CTCs were re-analysed immediately after the disease was judged as progressive; no CTCs were detected in any of these cases.

Correlation between CTC number and therapeutic outcome. As stage IV CRC is a systemic disease, we considered that CTCs may be more prevalent in this stage. However, CTCs were not always detected, even in stage IV cancer. To determine whether the presence of CTCs is related to the therapeutic outcome, we analysed the relationship between the number of CTCs and the time to therapeutic failure (TTF) of chemotherapy administered when CTCs were counted. The number of CTCs and TTF are shown in Table II. Chemotherapeutic agents included an oxaliplatin-based regimen with or without bevacizumab (BV) (n=5), an irinotecan-based regimen (n=5), 5-fluorouracil (5-FU) plus leucovorin (n=1), and no therapy (n=1). In the latter case, time to progression (TTP) was applied. The Pearson product-moment correlation coefficient was calculated. A negative correlation was observed between the number of CTCs and the therapeutic outcome, but this relationship was not significant ($y=4.71-0.0076x$; correlation coefficient=–0.3897; $p=0.21$) (Figure 1).