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.

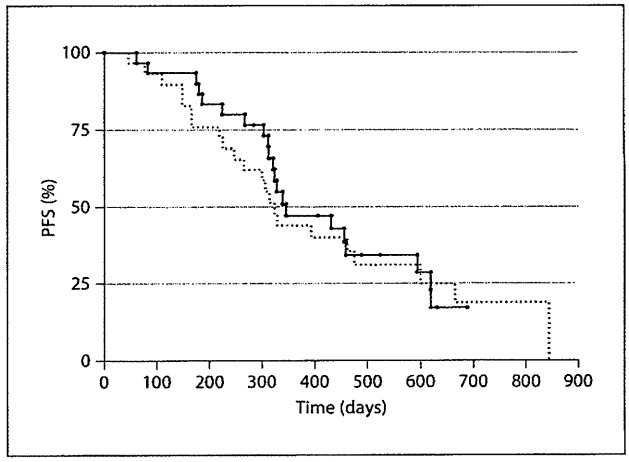

Fig. 1. Kaplan-Meier PFS curves of patients with metastatic colorectal cancer treated with mFOLFIRI + bevacizumab (dotted line) and IRIS + bevacizumab (solid line).

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

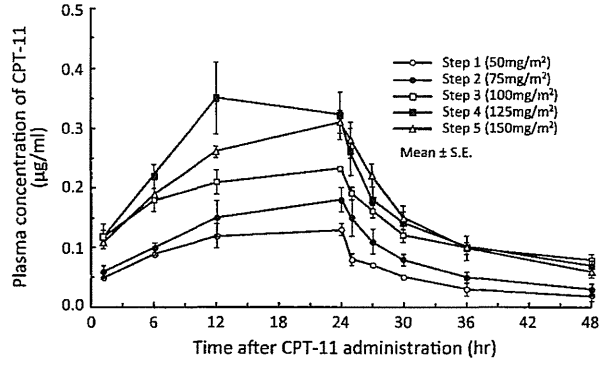

Fig. 1 Mean plasma concentrations of irinotecan (CPT-11) after drip infusion of CPT-11 and 5-fluorouracil (5-FU) (800 mg/m^2) in humans

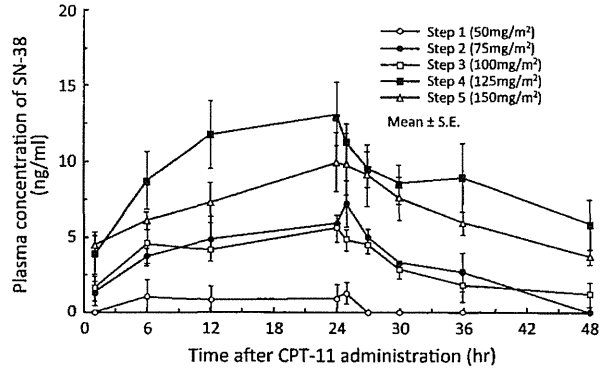

Fig. 2 Mean plasma concentrations of SN-38 after drip infusion of CPT-11 and 5-FU (800 mg/m^2) in humans

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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CASE REPORT

The effect of XELOX plus bevacizumab on rectal hepatoid adenocarcinoma

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Abstract Colorectal hepatoid adenocarcinoma is rarely reported and is known to have a poor prognosis. Reports of chemotherapy against colorectal hepatoid adenocarcinoma are scarce. Here, we provide the first report of a case involving XELOX + bevacizumab treatment of rectal hepatoid adenocarcinoma. The patient, a 40-year-old female, was diagnosed with early rectal cancer and underwent endoscopic mucosal resection. Fourteen months later, multiple lymph node metastases appeared. She received XELOX + bevacizumab, and maintained stable disease for approximately ten months. However, unfortunately, this regimen had to be stopped because of interstitial pneumonitis. She underwent other chemotherapies and chemoradiotherapies but died approximately two years after the recurrence. Our results indicate that the XELOX + bevacizumab regimen may be effective for controlling this disease.

Keywords Colorectal hepatoid adenocarcinoma · Alpha-fetoprotein · XELOX · Bevacizumab

Introduction

Hepatoid adenocarcinoma (HAC) is a rare extrahepatic neoplasm that is characterized by morphologic phenotypes similar to those of hepatocellular carcinoma (HCC) [1, 2]. The term HAC was coined for a tumor of the stomach by Ishikura et al. [3]. It has mostly been reported to occur in the stomach (63 %), ovary (10 %), lung (5 %), gallbladder (4 %), pancreas (4 %), uterus (4 %), and rarely in the colorectum (2 %) [4]. We searched the PubMed online database for colorectal HAC (CRC-HAC) and found only 12 case reports [5–16]. According to these few reports, this is a rare disease characterized by poor prognosis. Standard regimens for CRC are those usually applied for adenocarcinomas, which are observed in over 90 % cases of CRC [17]. However, there is still controversy regarding the agents that should be applied for CRC-HAC (for example, standard chemotherapies or chemotherapies for HCC, such as sorafenib). It is also reported that regimens for germ cell tumors have been applied for gastric HAC due to its alpha-fetoprotein (AFP) production. Furthermore, as the number of published CRC-HAC cases is limited, the reported regimens applied for these diseases are somewhat old-fashioned, such as 5-fluorouracil (5-FU) alone (Table 1). One report has described the effect of agents such as oxaliplatin and irinotecan [16]. In this paper, we report the effectiveness of recently approved agents such as capecitabine, S1, bevacizumab, and panitumumab (P-mab).

Case report

A 40-year-old female presented to a clinic with bloody feces. She underwent a total colonoscopy that revealed a 7-mm-diameter subpedunculated tumor in the rectum (Fig. 1a). The tumor was resected by EMR. The patient's medical history revealed that she was diagnosed with Turner syndrome with mosaicism of 45,X/46,X,del(Xp) in her twenties. Histopathological findings showed that the tumor consisted of areas of both well-differentiated tubular

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Table 1 Therapeutic outcomes of CRC-HAC in the literature

Author	Year	Age	Gender	Primary	Metastasis	Histology	AFP (ng/ml)	Treatment	Survival (M)
Nakajima	1985	50	M	R	Liver	Ad (por, mod), SCC	3018	Surg, HAI	6 (S)
Sato	1994	43	M	R	Liver	Ad (mod)	941	Surg, HAI, Cx (5-FU), IFN	3 (S)
Taguchi	1997	71	M	R	Liver	Ad (well), medullary	220000	Surg, HAE	12 (S)
Yachida	2003	59	M	T	Liver	Ad (pap, tub)	12873	Surg, Cx (5-FU, LV)	2 (S)
Fu	2006	71	M	Ce	LN	Ad (por, mod)	319	Surg, Ad-Cx	60 < (alive) (S)
Borgonovo	2008	42	M	R	Liver, LN	Hepatoid	32000	Surg, HAE	18 (S)
Cappetta	2012	75	F	A	LN, (PC, PLC)	Ad (por)	9	Surg, Cx (FOLFOX, FOLFIRI)	8 (S)
Otsuka		40	F	R	LN, (PLC, lung)	Ad (well), Hepatoid	1194	EMR, Cx (Xelox + BV, S1, IRI, P-mab	24 (Re)

Metastatic sites in parentheses were found at fur advanced stage

R rectum, *T* transverse colon, *Ce* cecum, *A* ascending colon, *LN* lymph node, *por* poorly differentiated, *mod* moderately differentiated, *pap* papillary, *Ad* adenocarcinoma, *Surg* surgical, *HAI* hepatic arterial infusion, *HAE* hepatic arterial embolization, *Cx* chemotherapy, *LV* leucovorin, (*S*) after surgical treatment, (*Re*) after recurrence, *PC* peritonitis carcinomatosa, *PLC* pleuritis carcinomatosa

adenocarcinoma and poorly differentiated adenocarcinoma (Fig. 1b). The cancer had invaded partly into the superficial layer of the submucosa. The depth of invasion was classified as submucosal invasion (SM1) and the margin was tumor-free. Fourteen months later, a follow-up computed tomography (CT) scan showed multiple swollen lymph nodes from the periaortic to the presacral regions (Fig. 2a, b), and the patient was subsequently admitted to our hospital. ^{18}Fluorodeoxyglucose-positron emission tomography (^{18}FDG-PET) revealed an abnormally increased uptake in multiple lymph nodes, including those in not only periaortic and presacral regions but also the left subclavicular and paraesophageal regions (Fig. 2c, d). As the tumors were possibly a cancer of unknown primary, various serum tumor markers were examined, and she showed elevated serum levels of AFP, carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA19-9). The levels of the serum AFP, CEA, and CA19-9 were 1194.2 ng/ml, 48.1 ng/ml, and 38.9 U/ml, respectively. Because of the unusually elevated AFP levels, we performed an immunohistochemical examination of the archival EMR specimen. The tumor was composed of two different types of cancer cells: one type was well-differentiated tubular adenocarcinoma, while the other was poorly differentiated solid adenocarcinomas with slightly eosinophilic cytoplasm and enlarged nuclei, characteristic of hepatoid adenocarcinomas. The poorly differentiated adenocarcinomas were AFP-positive. These cells were considered to be possible hepatoid adenocarcinomas (Fig. 1c). As a result, we diagnosed multiple lymph node metastasis derived from the resected AFP-producing rectal cancer. The detailed diagnostic process in this case has already been reported elsewhere in Japanese. Although there are no established regimens for CRC-HAC, based on the previous reports, the patient was treated with chemotherapy with an oxaliplatin plus capecitabine (XELOX) + bevacizumab (BV) regimen (oxaliplatin, 130 mg/m^2 on day 1; capecitabine, 2000 mg/m^2 on days 1–14; BV, 7.5 mg/kg on day 1, every three - weeks) for up to 14 courses. Several CT examinations over the next ten months showed that the metastatic lymph nodes were approximately the same size, and that no new lesions had appeared. This patient was categorized as having stable disease (SD) according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria (version 1.1) (Fig. 2e, f). However, the patient developed grade 2 interstitial pneumonitis, as defined by the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 scale. In lieu of these findings, we discontinued the chemotherapy regimen, although other adverse events such as hypertension (grade 2), nausea (grade 2), and urticaria (grade 2) were tolerable. The patient's treatment regime was changed to concurrent chemoradiotherapy (CRT) with S-1 (tegafur, gimeracil, and oteracil potassium, 50 Gy/25 fractions for periaortic lymph node metastasis), followed by single therapies with S-1 and irinotecan (IRI), one after the other. Furthermore, P-mab therapy was initiated, because the tumor cells contained wild-type codons 12 and 13 of the *KRAS* gene. However, the disease was not controlled by any treatment other than XELOX + BV or CRT with S-1. The patient's serum AFP level decreased below 500 ng/ml during XELOX + BV or CRT with S-1 therapies. Similarly, CEA and CA19-9 decreased to normal levels (20.7 and 13.7 ng/ml, respectively) with these treatments. The second treatment with CRT (to the

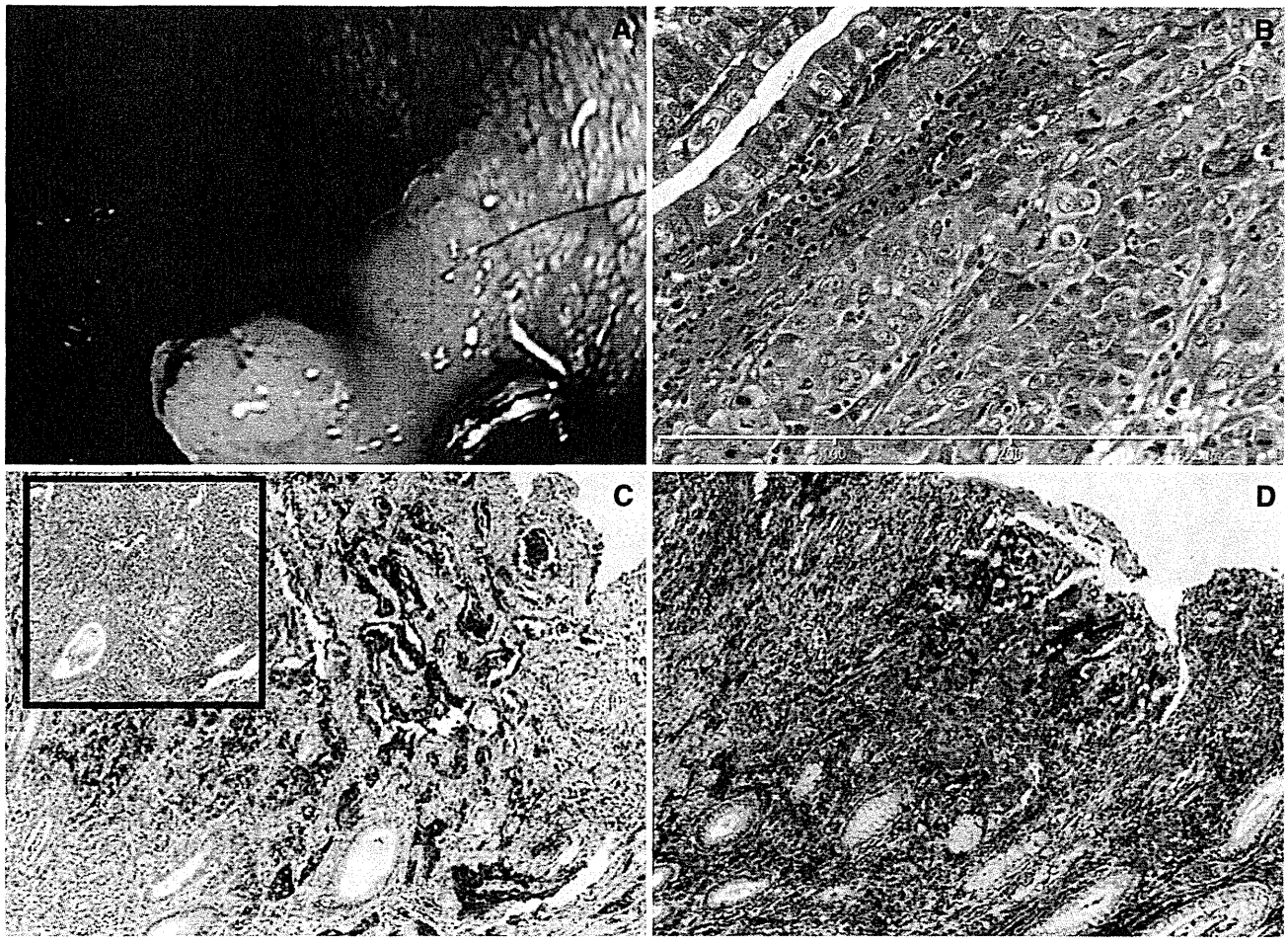

Fig. 1 Clinicopathological features of the rectal tumor. **a** Endoscopic view. **b** Histopathological findings for the EMR specimen. The tumor consisted of both well-differentiated tubular adenocarcinomas and poorly differentiated solid adenocarcinomas with slightly eosinophilic cytoplasm and enlarged nuclei, characteristic of hepatoid adenocarcinomas. **c** Immunohistochemical staining of the rectal tumor for AFP. Immunohistochemical analysis showed that the poorly differentiated adenocarcinomas were AFP-positive. The *inset* indicates H&E staining of the same lesion. **d** Immunohistochemical staining for thymidine phosphorylase

presacral regions and the left subclavicular lymph nodes) and S-1 also decreased the serum AFP level; however, the response remained within the SD criteria. The other treatments did not decrease the serum AFP level any further, or the CEA and CA19-9 levels (Fig. 3). The patient had bulky lymph node metastases but no blood-borne metastases for 21 months after her relapse. Twenty-four months after the recurrence, the patient died of multiple lung metastases.

Discussion

Colorectal hepatoid adenocarcinoma is rare, and reports of chemotherapy for its advanced stage are limited. Only seven studies describing chemotherapy have been reported (Table 1), and four of them described hepatic arterial infusion or embolization against liver metastasis, as most of the reported cases showed hematogenous spread. Lymphogenous spread is not typical [14], but it has been mentioned more often in recent publications than in those published previously. Around 2003, 5-fluorouracil (5-FU) was used in systemic chemotherapy of CRC-HAC. A recent publication described the combined therapy of 5-FU with oxaliplatin or IRI, which is standard for general CRC, in which a lower survival rate (eight months) was achieved than the usual median survival time (MST) of CRC. It is still unclear whether CRC-HAC is resistant to the standard regimens for CRC. It was reported that sorafenib, approved for advanced HCC, was used in one case of AFP-producing adenocarcinoma in the peritoneal cavity—an HAC—in which the patient survived for six months [18]. It was also reported that regimens similar to those used for germ-cell tumors were applied in two gastric HAC cases [19]. The treatments were rather effective, but relatively toxic, as shown by the therapy-related death in one case. There are no established regimens for advanced HAC of digestive

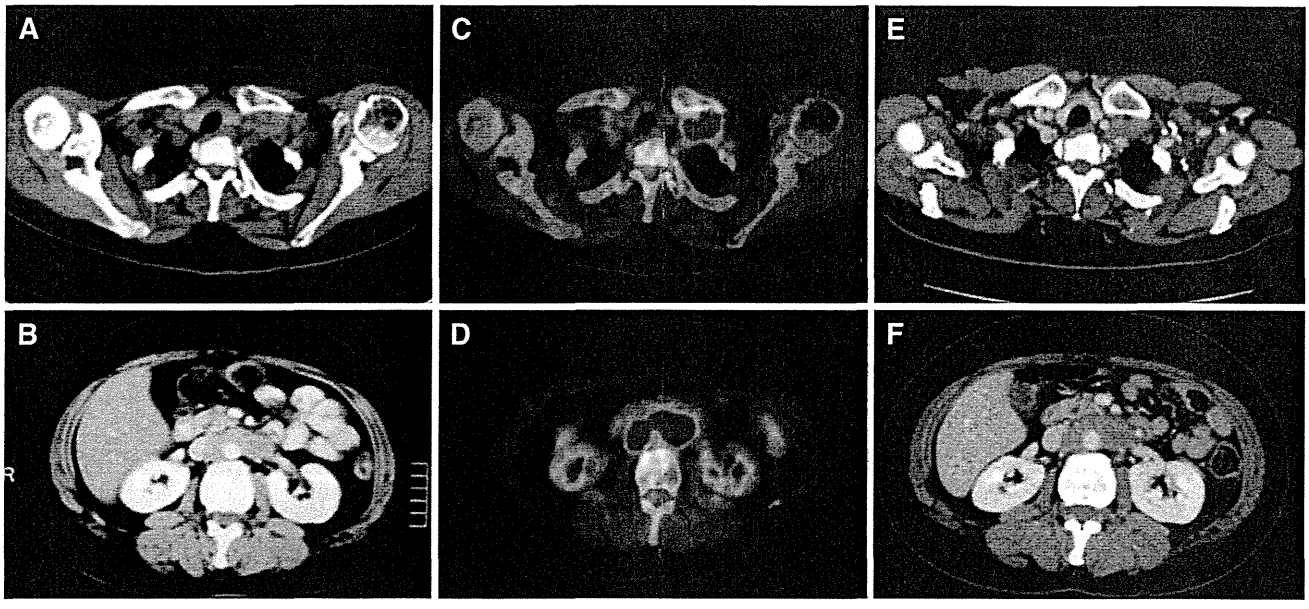

Fig. 2 Imaging of the lymph node metastases. **a** CT imaging of the subclavian lymph nodes before treatment. **b** CT imaging of the bulky paraaortic lymph nodes, same as above. **c** ^{18}FDG uptake in the subclavian lymph nodes before treatment. **d** ^{18}FDG uptake in the bulky paraaortic lymph nodes, same as above. **e** CT imaging of the subclavian lymph nodes after five cycles of XELOX + BV treatment. **f** CT imaging of the bulky paraaortic lymph nodes, same as above

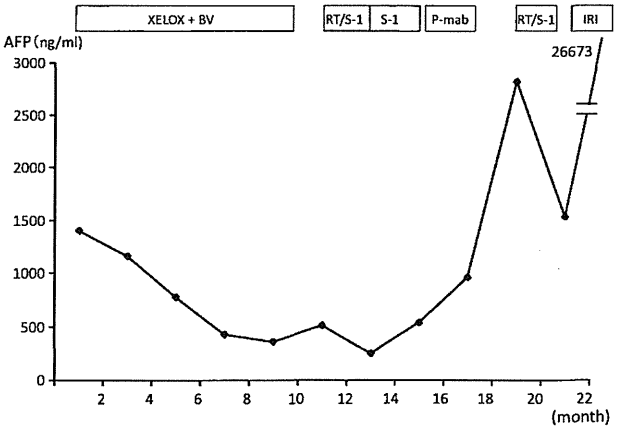

Fig. 3 Change in AFP serum level during treatment. Months after recurrence are indicated

organs except for HCC. In the present report, current standard regimens for advanced CRC, including two molecular-targeted antibodies, were applied. Survival time after recurrence was 24 months, which was comparable to the MSTs associated with typical CRC therapies. Individually, XELOX + BV and CRT with S1 were effective, but IRI and P-mab were not. Thymidine phosphorylase (TP) is upregulated in a wide variety of solid tumors, including colorectal cancer, and it stimulates tumor growth and is associated with poor prognoses [20]. TP is indispensable for the activation of capecitabine, which is converted to 5-FU in the tumor [18]. Indeed, there are some reports which have indicated that high TP expression levels are associated with the response of the tumor to capecitabine [21, 22]. TP expression was confirmed as it was shown experimentally that TP expression was suppressed in an AFP-producing adenocarcinoma cell line [23]. In the present case, immunohistochemical staining for TP was positive in the initial tumor (Fig. 1d). Actually, this tumor was relatively sensitive to the XELOX regimen. This case indicated that standard CRC regimens can control advanced CRC-HAC. This patient also suffered from Turner syndrome. In a study of cancer incidence in a cohort of 597 women with Turner syndrome, the relative risk of CRC was rather high (6.9) [24]. However, the relationship between CRC-HAC and Turner syndrome is unclear, and there are no reports on CRC-HAC combined with Turner syndrome in the literature.

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Conflict of interest The authors declare that they 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).

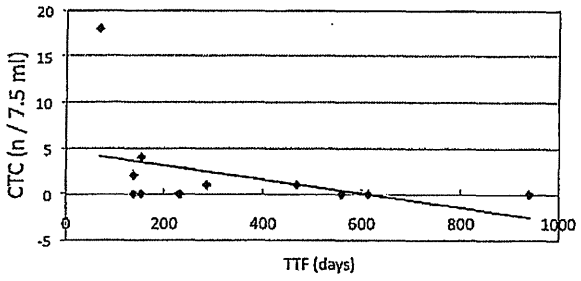

Figure 1. *Correlation between the number of circulating tumor cells (CTCs) during therapy and time to treatment failure (TTF).*

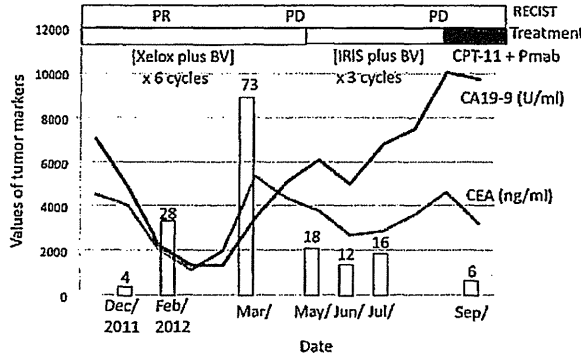

Figure 2. *Change in number of circulating tumor cells (CTCs) during the treatment of case 3. The number of CTCs is indicated by white bar. Carcinoembryonic antigen (CEA) is indicated in blue and carbohydrate antigen 19-9 (CA19-9) in red. The result of response evaluation criteria in solid tumors (RECIST) in each timing is indicated at the top. Xelox, Capecitabine + oxaliplatin; BV, bevacizumab; IRIS, irinotecan + S1; CPT-11, irinotecan; Pmab, panitumumab; PR, partial response; PD, progress disease.*

Table II. *Number of circulating tumor cells detected during therapy and treatment outcome.*

Case	CTC (n/7.5 ml)	TTF (days)
1	0	940 (FOLFOX+BV)
2	2	139 (Xelox)
3	4	153 (Xelox+BV)
4	18	69 (IRIS+BV)
5	0	232 (CPT-11+Pmab)
6	1	468 (FOLFIRI+Pmab)
7	0	139 (SOX)
8	0	230 (SOX)
9	0	613 (FOLFIRI+BV)
10	0	153 (CTP-11+Cmab)
11	0	559 (FL)
12	0	287 (no therapy, TTP)

TTF, time to treatment failure; FOLFOX, 5-fluorouracil + leucovorin + oxaliplatin; BV, bevacizumab; Xelox, Capecitabine + oxaliplatin; IRIS, irinotecan + S1; Pmab, panitumumab; FOLFIRI, 5-fluorouracil + leucovorin + irinotecan; SOX, S1 + oxaliplatin; CPT-11, irinotecan ; FL, 5-fluorouracil + leucovorin, TTP, time to progression.

Potential use of CTCs as a predictive biomarker for outcome of chemotherapy for CRC. Comparison of the number of CTCs before and after chemotherapy could predict the treatment outcome. In case 3, we detected CTCs several times. We compared the change in CTC number with other evaluative methods, such as Response Evaluation Criteria in Solid Tumors (RECIST) and the tumour markers carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9). As shown in Figure 2, an increase in the number of CTCs was observed during Xelox plus BV treatment, three months prior to RECIST evaluation, and one month prior to the increase in tumour markers. The same trend was observed for treatment with irinotecan plus S1 (IRIS) plus BV. In case 13, the number of CTCs declined from 1 to 0 during capecitabine plus oxaliplatin (XELOX) therapy. A decrease in CTCs was associated with partial response (PR; RECIST) evaluated at two-month intervals beginning with the initiation of therapy and was also associated with a decrease in tumour markers between the baseline measurement and during therapy of CEA (from 1636.1 to 187.5 ng/ml) and CA19-9 (from 2137.0 to 411.8 U/ml). The number of CTCs did not increase for four months, and the disease kept within stable disease (SD; RECIST) criteria during this period. These observations demonstrate that if CTCs are detectable, changes in the number present after treatment may be useful for predicting therapeutic outcomes much earlier than that with the current methods.

In the cases where CTCs were not present initially, they were not detected even after the disease progression (cases 1, 4, and 11; Table I). In the CTC-negative cases, we did not obtain any predictive values.

Utility of CTCs as a sample source for molecular analysis. We attempted to analyse *KRAS* in the DNA derived from CTCs collected in cases 2, 3 (twice), 5, 12, and 13 using the Scorpion-ARMS method. No DNA was amplified in case 3 or case 12, where the number of CTCs was 4 and 1 per 7.5 ml of whole blood, respectively (Table III). In the other four cases, where the number of CTCs ranged from 1 to 28 per 7.5 ml of whole blood, the DNA was insufficiently amplified, and no *KRAS* mutants were amplified. For cases 3 and 13, we compared the results of Scorpion-ARMS analysis from surgically removed tissue samples and CTCs. While analysis of the tissue samples identified both cases as having