TABLE 2 Toxicity

Toxicity	SWIFT 1: FOLFOX4 (n=54)				SWIFT 2: mFOLFOX6 (n=58)			
	No. of patient (%)				No. of patient (%)			
	G2	G3	G4	G3 and 4	G2	G3	G4	G3 and 4
Leucocytopenia	12(22.2%)	11(20.4%)	0	11(20.4%)	24(41.4%)	3(5.2%)	1(1.7%)	4(6.9%)
Neutropenia	11(20.4%)	18(33.4%)	10(18.5%)	28(51.9%)	16(27.6%)	19(32.8%)	7(1.2%)	26(44.8%)
Anemia	10(18.5%)	0	0	0	18(31.0%)	2(3.5%)	0	2(3.5%)
Thombocytopenia	9(16.7%)	2(3.7%)	0	2(3.7%)	9(15.5%)	1(1.7%)	0	1(1.7%)
AST elevation	9(16.7%)	1(1.9%)	0	1	7(12.1%)	0	0	0
ALT elevation	3(5.6%)	2(3.7%)	0	2	8(13.8%)	2(3.5%)	0	2(3.5%)
Anorexia	8(14.8%)	0	0		11(19.0%)	6(10.3%)	0	6(10.3%)
Nausea	2(3.7%)	0	0		5(8.6%)	4(6.9%)	0	4(6.9%)
Vomiting	2(3.7%)	1(1.9%)	0		2(3.5%)	2(3.5%)	0	2(3.5%)
Diarrhea	0	1(1.9%)	0	1	2(3.5%)	1(1.7%)	0	1(1.7%)
Stomatitis	1(1.9%)	1(1.9%)	0	1	3(5.2%)	0	0	0
Hand-foot syndrome	0	0	0	0	2(3.5%)	1(1.7%)	0	1(1.7%)

)urse in five patients (11.1%) in the SWIFT-1 eries and in 17 patients (36.9%) in the SWIFT-2 eries. Grade 2 or 3 neuropathy frequently devel- ped in the fourth or later courses. No difference the frequency of Grade 2 or 3 neuropathy was bserved between the SWIFT1 and SWIFT2 se- es. The relative dose intensities (RDI) in this trial ere 81.9% for oxaliplatin, 83.2% for bolus 5FU, nd 81.8% for infusion 5FU in SWIFT1, and 82.1%, 4.1%, and 84.4%, respectively, in SWIFT2 (**Table**). Ten patients (18.2%) were withdrawn from the udy because of adverse events in SWIFT1 and 14 4.1%) were withdrawn because of adverse events SWIFT2.

fficacy

Overall, out of 112 evaluable patients, the me- an number of treatment courses was 7.5 (range, 1 19 courses) in SWIFT1 and 8 (range, 1 – 16 cours-) in SWIFT2. The objective responses are listed **Table 5**. Three patients had complete responses Rs), and 53 patients had partial responses (PRs; tal responses, 56/112 [50.0%]). Forty-four patients had stable diseases (SDs; 44/112 [39.3%]), and 5 patients had progressive diseases (PDs; 5/112: [4.5%]); 6 patients could not be evaluated (NE; 6/112 [5.4%]). The objective response rate was 50.0% (95% CI, 27.1% to 54.6%) for SWIFT-1&2. In the SWIFT1 series, the antitumor efficacy rating was CR, PR, SD, and PD in 1.9%, 51.9%, 37%, and 3.7% of the patients, respectively, with a response rate (CR + PR: 30/54) of 55.6%. In the SWIFT2 series, the antitumor efficacy rating was CR, PR, SD, and PD in 3.4%, 43.1%, 41.4%, and 5.2% of the patients, respectively, with a response rate (CR + PR: 27/58) of 46.6%. The response rates (CR + PR) according to metastatic site were 54.1% (46/85) for the liver, 17.4% (4/23) for the lung, and 23.3% (7/30) for the lymph nodes in SWIFT-1&2 (**Table 6**). The median progression-free survival time was 9.0 months in SWIFT-1 and months in SWIFT-2 (**Figure 3**). The median survival time was 21.5 months in SWIFT-1 and 21.6 months in SWIFT-2 (**Figure 4**).

DISCUSSION

In Western countries, the standard chemother-

TABLE 3 Neurologic Toxicity

Neurologic toxicity	SWIFT 1: FOLFOX4 (n=54)				SWIFT 2: mFOLFOX6 (n=58)			
	No. of patient (%)				No. of patient (%)			
	G1	G2	G3	G4	G1	G2	G3	G4
NCI-CTCA	26 (48.1%)	10 (18.5%)	3 (5.6%)	0	29 (50%)	14 (24.1%)	3 (5.2%)	0
OEB-NTC	23 (42.6%)	12 (22.2%)	3 (5.6%)	0	24 (41.4%)	22 (37.9%)	2 (3.5%)	0

NCI-CTCA: V3.0
OEB-NTC: oxaliplatin-specific scale

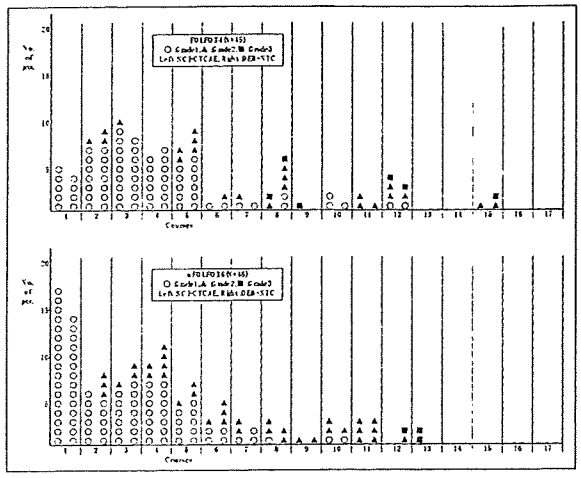

FIGURE 2
Appearance of neuropathy

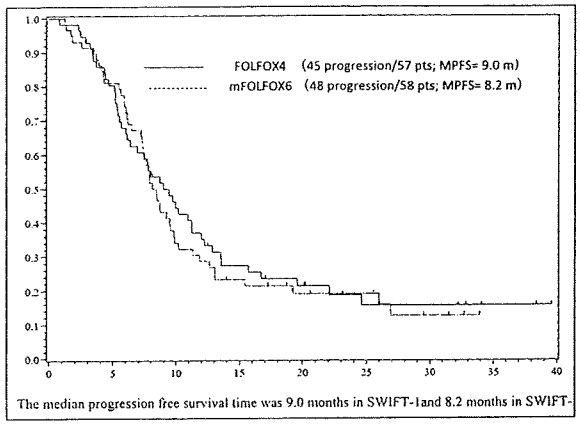

FIGURE 3
Progression-free survival rate of all enrolled patients. The median progression-free survival time was 9.0 months in SWIFT-1 and 8.2 months in SWIFT-2

apy regimens for the treatment of advanced colorectal cancer are L-OHP + 5FU/LV (FOLFOX4 and mFOLFOX6 regimens)(11,16,17), CPT-11 + 5FU/LV (FOLFIRI and AIO regimens), and additional molecular-targeting therapies. The median survival time (MST) after these therapies currently exceeds 20 months. In Japan, 5FU/LV has been recognized as a treatment for advanced colorectal cancer since l-LV was approved in 1999. When CPT-11 and L-OHP were developed (18, 19), their clinical trials were conducted in combination with 5FU/LV. Later, a controlled trial of LV/5FU2 versus L-OHP versus FOLFOX4 was conducted in patients who were resistant to IFL therapy, which was the standard treatment for advanced colorectal cancer in the U.S. The study reported that the FOLFOX regimen was significantly superior (16). Subsequently, a phase III randomized controlled clinical trial showed that

TABLE 4 Relative Dose Intensity

	SWIFT-1	SWIFT-2
	FOLFOX4	mFOLFOX6
oxaliplatin	81.9%	82.1%
5FU (bolus)	83.2%	84.1%
Infusional 5FU	81.8%	84.4%

combination therapies including CPT-11 or L-OHP had a much better response rate and progression-free survival period than 5FU/LV (12, 14, 17, 20). Thus, these combinations replaced 5FU/LV as the standard systemic treatments for metastatic advanced colorectal cancer.

In Japan, infusion 5FU/LV was approved in February 2005, and the FOLFOX therapy became available at that time. However, no phase II trial had been conducted in Japanese patients with advanced colorectal cancer examining combination therapies using FOLFOX4 or mFOLFOX6 at that time. Thus, a multicenter phase II clinical trial was conducted to examine the feasibility of these regimens in actual clinical practice. This trial included a total of 112 cases with evaluable lesions treated using either the FOLFOX4 regimen (SWIFT-1, n=54) or the mFOLFOX6 regimen (SWIFT-2, n=58). As for the treatment results, the overall response rate was 50.0% (53.7% in SWIFT-1 and 46.6% in SWIFT-2), the overall MST was 21.5 months (21.5 months in SWIFT-1 and 21.6 months in SWIFT-2), and the overall progression-free survival (PFS) period was 8.7 months (9.0 months in SWIFT-1 and 8.2 months in SWIFT-2). These results were comparable to those reported in Western studies (**Table 7**) (10, 11, 21-23) and in the study by Shimizu *et al.* (24), and no significant differences were observed between SWIFT-1 and SWIFT-2. In this trial, many patients had liver metastasis, and the response rate in the patients with liver metastasis was 54.1%, which was the highest value among the patient groups according to metatastic site. This finding strongly suggests that the L-OHP + 5FU/LV regimen is effective and useful as an initial therapy in patients with liver metastasis.

As for adverse reactions, the incidences of grade 3 or higher adverse events were 13.4% for leukopenia, 48.2% for neutropenia, and 1.8% for anemia. These results were also comparable to those reported in Western studies. The mean number of courses

TABLE 5 Response

	CR	PR	SD	PD	NE	Response rate
	No. of patient (%)					
SWIFT-1: FOLFOX4	1(1.9%)	28(51.9%)	20(37%)	2(3.7%)	2(3.7%)	29/54 (53.7%)
SWIFT-2: mFOLFOX6	2(3.4%)	25(43.1%)	24(41.4%)	3(5.2%)	4(6.9%)	27/58 (46.6%)
SWIFT-1&2	3(2.7%)	53(47.3%)	44(39.3%)	5(4.5%)	6(5.4%)	56/112 (50.0%)

administered to the subjects was 7.5 (range, 1 – 19 courses) in SWIFT-1 and 8 (range, 1 – 16 courses) in SWIFT-2. In the SWIFT-1 series, the incidence of grade 2 and grade 3 peripheral sensory neuropathy, a characteristic adverse reaction of L-OHP, was 18.5%/5.6% according to the NCI-CTCAE criteria and 22.2%/5.6% according to the DEB-NTC criteria; in the SWIFT-2 series, the respective incidences were similar: 24.1%/5.2% and 37.9%/3.5%, respectively. In Western countries, the incidence of grade 2 and 3 peripheral neurotoxicity was reported as 29.2%/18.2% after FOLFOX4 therapy used as an initial therapy. Although a direct comparison is not appropriate, these results are almost comparable to those for the SWIFT-1 and SWIFT-2 series.

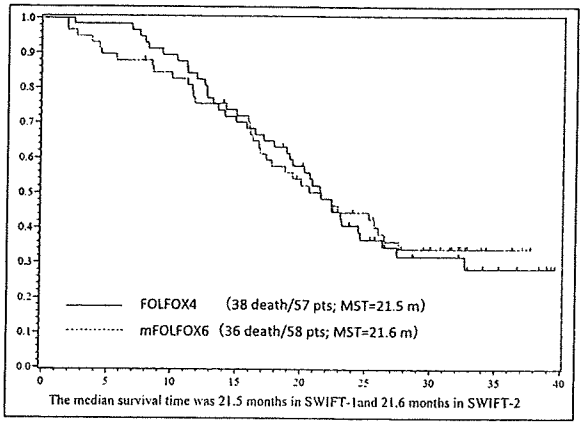

FIGURE 4 Overall survival rate of all enrolled patients. The median survival time was 21.5 months in SWIFT–1 and 21.6 months in SWIFT–2

TABLE 6 Response by metastatic sites

Site of metastases	Total: SWIFT-1&2		FOLFOX4: SWIFT-1		mFOLFOX6: SWIFT-2	
	No. of patient (%)		No. of patient (%)		No. of patient (%)	
	CR+PR/n	Response rate(%)	CR+PR/n	Response rate(%)	CR+PR/n	Response rate(%)
Liver	46/85	54,1	24/43	67,4	22/35	80
Lung	4/23	17,4	5/6	83,3	3/7	42,9
Lymph node	7/30	23,3	3/9	33,3	3/13	23,1

TABLE 7 Comparison of other studies

	FOLFOX4 in First-Line				mFOLFOX6 in First-Line		
	SWIFT1 present study	C95-1 de Gramont[1]	N9741 Goldberg[2]	OPTIMOX1 Tournigand[3]	SWIFT2 present study	OxMdG Cheeseman[4]	FOCUS Seymour[5]
No. of patients	54	210	267	311	58	25	299
Age, years							
Median	62	63	61	65	63	62	64
Range	25-74	20-76	27-88	29-80	25-75	14-77	56-69
PS, %							
0	77.8	43.3	] 93	52	77.6	40	41
1	20.4	46.2		] 48	22.4	44	50
2			5			16	8
Metastatic site, %							
Liver	81.5	86.7	unknown	71	70.7	unknown	unknown
Lung	22.2	23.4	unknown	26	19	unknown	unknown
Other	27.8	12.4	unknown	10	29.3	unknown	unknown
Adjuvant Chemotherapy, %	24.1	20	16	22	20.7	24	unknown
RR, %	55.6	50.7	45	58.5	46.6	72	56.2
PFS, months	9.4	9	8.7	9	8.5	10.6	9.1
OS, months	20.2	16.2	19.5	19.3	21.6	16.7	15.2

Grade 1 peripheral sensory neuropathy developed during the first treatment course, and the frequency of this complication was similar in the SWIFT-1 and SWIFT-2 series. However, grade 2 and 3 peripheral nerve disorders frequently developed during the fourth or later courses. The grade of the disorder was higher for later treatment courses, as reported by de Gramont *et al.* (11). The relative dose intensities (RDI) in this trial were 81.9% for L-OHP, 83.2% for bolus 5FU, and 81.8% for infusion 5FU in SWIFT-1, and 82.1%, 84.1%, and 84.4%, respectively, in SWIFT-2. Factors responsible for RDI reductions included hemotoxicity (leukopenia, neutropenia, and thrombocytopenia) and peripheral nerve disorders in the both studies (25). The response rate, PFS, MST and safety of the FOLFOX4 and mFOLFOX6 combination therapies were equivalent in our multicenter phase II clinical trial and were somewhat better than those reported in foreign trials.

This clinical trial demonstrated that FOLFOX therapy is as effective and safe in Japanese patients with unresectable advanced colorectal cancer as it is in patients in foreign countries and may be remarkably effective if used in general practice in Japan. However, since the manifestations of peripheral sensory neuropathy increase with increases in the number of doses, future treatment strategies require preventive measures to ensure that the QOL of these patients is not reduced.

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A Feasibility Study of UFT/LV and Irinotecan (TEGAFIRI) in Advanced or Metastatic Colorectal Cancer: Osaka Gastrointestinal Cancer Chemotherapy Study Group (OGSG) PROG 0304

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Objective: This is a feasibility trial of oral uracil/tegafur (UFT)/oral leucovorin (LV) and irinotecan (TEGAFIRI) with maximum dose confirmed in Japan. To document the toxicity and define the objective response rate (RR); and determine progression-free and overall survival.
Methods: Patients with advanced or metastatic colorectal cancer (CRC) received: UFT 300 mg/m^2, LV 75 mg/body and CPT-11 150 mg/m^2 (UFT and LV given on days 1–14, and CPT-11 on day 1, every 3 weeks). Eligibility: ECOG performance status (PS) 0–1, adequate bone marrow/liver function and serum creatinine level less than institutional normal value.
Results: Eighteen patients enrolled, 17 evaluable for toxicity and response and 1 patients recalled chemotherapy upon registration. Characteristics: 61% male, median age 63.5 years (51–71). Seventy-two per cent PS 0, 50% first line. One hundred and eighty-six cycles have been delivered. The common Grade 3–4 toxicities were neutropenia (35.3%), leukopenia (29.4%), diarrhea (5.9%), anorexia (5.9%), vomiting (5.9%) and dizziness (5.9%). There was no episode of febrile neutropenia. No death occurred on treatment: Overall RR was 41.2% [7/17: 1 complete response (CR) + 6 partial response (PR)]. Progression-free survival (PFS) is 6.9 months, median survival time (MST) is 25.1 months and 1-year survival rate is 70.6%, whereas PFS 15.0 months, MST 43.6+ months and 1-year survival rate 100% in cases with CR or PR.
Conclusions: Approved dose of CPT-11 is 150 mg/m^2 in Japan. As is lower dose with CPT-11, TEGAFIRI for patients with advanced or metastatic CRC in Japan seems to have the similar effect with that reported abroad and indicates prolonged PFS and MST in cases with CR or PR.

Key words: colorectal cancer – chemotherapy – TEGAFIRI

INTRODUCTION

Combination chemotherapy of oxaliplatin and 5-fluorouracil (5-FU)/leucovorin (l-LV) (FOLFOX) or combination chemotherapy of CPT-11 and 5-FU/l-LV (FOLFIRI) has been used as standard regimens for advanced or metastatic colorectal cancer (CRC) in Japan. However, both regimens may have damage for patients' quality of life, because continuous infusion of 5-FU needs operation making central venous route or short hospitalization.

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It is reported that oral capecitabine had a strong trend for better survival than intravenous 5-FU/l-LV (1,2), and oral uracil/tegafur (UFT) plus oral leucovorin (LV) had the same survival as 5-FU–LV (3–5). Furthermore, combination chemotherapy of oxaliplatin and capecitabine is reported to be as effective as FOLFOX (6–8), combination chemotherapy of oxaliplatin and UFT/LV as FOLFOX (9), combination chemotherapy of CPT-11 and capecitabine as FOLFIRI (10), combination chemotherapy of CPT-11 and UFT/LV as FOLFIRI (9,11,12), whereas only UFT/LV and irinotecan (TEGAFIRI) is approved in Japan.

Two clinical studies were presented in Osaka Gastrointestinal Cancer Chemotherapy Study Group at the

start of TEGAFIRI. One is Phase I/II study to explore the efficacy and safety in patients with advanced/metastatic CRC (protocol no. 0303) and the other is feasibility study to explore the efficacy and safety of TEGAFIRI reported abroad with maximum dose approved in Japan (protocol no. 0304). This is a final report of the latter study.

PATIENTS AND METHODS

Patients

This study was approved by respective Institutional Review Board. The subjects were patients with advanced or recurrent CRC who fulfilled the following conditions: a measurable lesion meeting the response evaluation criteria in solid tumors (RECIST) with no history of radiation therapy, an age of $\leq$75 years, an ECOG performance status (PS) of 0–1, adequate function of major organs and no prior therapy with CPT-11. Other prior therapy, if any, had to be ceased at least 4 weeks before the study to avoid a carry-over effect.

Treatment

Subjects received CPT-11 (150 mg/m^2) on day 1, UFT (300 mg/m^2) on days 1–14 and LV (75 mg/day) on days 1–14 of each 21-day cycle. A steroid (equivalent to 8 mg of dexamethasone) and a 5-HT3 receptor antagonist (antiemetic) were administered to prevent CPT-11-induced nausea and vomiting. Subjects were defined as completing per protocol treatment when the following conditions were fulfilled on day 1 of the third cycle: delay of CPT-11 therapy by $\leq$7 days, missed UFT/LV treatment for $\leq$7 days, disappearance of similar toxicities following dose reduction, no Grade 3–4 increase in GOT or GPT, and a PS $\leq$ 2. Subjects were defined as withdrawing from treatment in any of the following cases: when treatment could not be completed, when an adverse event made it difficult to continue treatment, when disease progression occurred and when the subject wished to discontinue therapy.

Evaluation

Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria (Version 3.0), and their incidence and severity were determined.

To assess the antitumor effect, the response rate (RR) was defined as the percentage of evaluable patients whose best overall response was classified as either CR or PR according to the RECIST (13).

The progression-free survival (PFS) was calculated as the time from the first day of treatment to the first day of documented progression or death.

The survival time was defined as the time from the day of registration to the final date of confirmed survival or the date of death.

Statistical Analysis

The present study was conducted to evaluate the rate of completing treatment when UFT/LV was used in combination with CPT-11. Assuming that the expected completion rate is 80%, the accuracy is 20% and the threshold completion rate is 60%, a minimum of 16 evaluable patients would be required. In consideration of this number and possible ineligible patients and/or dropouts, the target number of patients for the present study was set at 18.

The Mann–Whitney U test was used for comparison between two independent groups and the log-rank test was used for comparison of survival. All statistical tests were two-tailed and $P < 0.05$ was considered to indicate a significant difference.

RESULTS

Patient Characteristics

A total of 18 patients were enrolled in the study (Table 1). More than half of the patients were men (61%) and their ages ranged from 51 to 71 years. The PS was 0 in 72% of the patients and 50% had not received prior chemotherapy.

Table 1. Patient characteristics

Characteristics	
No. of patients	18
Age (years)	
Median	63.5
Range	51–71
Sex (%)	
Male	72.2
Female	27.8
ECOG performance status (%)	
0	61.1
1	38.9
Previous therapy (%)	
None	50
mFOLFOX6	5.6
5-FU derivatives	44.4
Tumor site (%)	
Colon	77.8
Rectum	22.2
Measurable lesions (%)	
Liver	44.4
Lymph nodes	38.9
Lung	11.1
Liver and lung	5.6

mFOLFOX, modified FOLFOX; 5-FU, 5-fluorouracil.

Fourteen patients had colon cancer (synchronous metastases in 10 patients and metachronous in 4 patients) and 4 patients had rectal cancer (synchronous metastases in 3 patients and metachronous in 1 patient) (patients who showed recurrence within 1 year of resection were classified as having synchronous metastasis).

There were measurable lesions of the liver in eight patients, lymph nodes in seven patients, lung in two patients, and both liver and lung in one patient.

Prior chemotherapy given within 6 months before the study was 5′-DFUR (doxifluridine) in three patients, UFT/LV in two patients, 5-FU/l-LV in one patient, S-1 (tegafur, gimeracil, oteracil potassium) in two patients and modified FOLFOX6 in one patient.

Treatment

One patient (63 years old with colon cancer for first-line treatment and a measurable lymph node metastasis) wished to change therapy after enrollment, so he received FOLFOX instead of TEGAFIRI. The remaining 17 patients received a total of 186 cycles of the present therapy (2–24 cycles per patient). Median dose intensity of CPT-11 was 83.8% and that of UFT was 81.1%.

One patient (a 66-year-old woman with rectal cancer for second-line treatment and a measurable lesion in the liver) did not complete therapy. The doses of CPT-11 and UFT were reduced because of Grade 3 leukopenia, Grade 3 neutropenia and Grade 3 anorexia, but similar adverse events occurred again. Therefore, treatment was discontinued on day 1 of the third cycle. Scheduled treatment could be continued in the remaining patients, so the treatment completions rate was 94.1% (16/17 patients).

One patient (a 58-year-old man with colon cancer for second-line treatment and a measurable lesion in the lung) underwent surgery. Because multiple nodules were observed in the lower lobe of the right lung during adjuvant chemotherapy, the patient selected chemotherapy first and the following operation if any other metastases were not seen in a few months. After completion of the second cycle, the response was rated as stable disease (SD), so curative resection was carried out at the patient's request.

From 6 to 24 cycles were administered to each responder, with a median number of 16 cycles. On the other hand, non-responders received two to eight cycles (except for a patient in whom the overall response was SD and 26 cycles were administered) and the median number of cycles for all non-responders was 5.

Subsequent chemotherapy was given to all 7 responders and 8 of the 10 non-responders. The percentage of responders undergoing subsequent treatment with FOLFOX was 57.1% (4/7 patients), whereas it was 71.4% for non-responders (5/7 patients, excluding 1 patient who had already received FOLFOX), and the rate was similar in the two groups ($P = 0.85$).

Toxicity

Dose reductions or treatment interruption for CPT-11 were needed for 29.4% of patients until day 1 of the third course and for 52.9% in all courses, and those for UFT were needed for none until day 1 of the third course and 29.4% in all courses.

Grade 3–4 adverse events (CTCAE Version 3.0) that occurred during treatment were neutropenia (35.3%), leukopenia (29.4%), diarrhea (5.9%), anorexia (5.9%), vomiting (5.9%) and dizziness (5.9%) (Table 2). There was no febrile neutropenia and no treatment-related death occurred.

Of the responders, only one experienced Grade 3–4 adverse events (Grade 3 leukopenia, Grade 4 neutropenia and Grade 3 diarrhea). In contrast, Grade 3–4 adverse events occurred in five non-responders, including three patients with SD and two patients with progressive disease (PD). There was no significant difference in the incidence of adverse events between responders and non-responders ($P = 0.29$).

Response

The best overall response was classified as CR in one patient, PR in six patients, SD in five patients, PD in four patients and not evaluable in one patient who underwent surgery. The RR was 41.2% (7/17 patients) (Table 3).

The RR achieved with first-line treatment was 37.5% (3/8 patients: 1 with CR and 2 with PR), whereas that for second-

Table 2. Frequency of common toxicities by the National Cancer Institute Common Toxicity Criteria (Version 3.0)

Toxicity	Highest grade/patient (%)		
	G0	G1 or G2	G3 or G4
Neutropenia	35.3	29.4	35.3
Leukopenia	41.2	29.4	29.4
Diarrhea	64.7	29.4	5.9
Anorexia	64.7	29.4	5.9
Vomiting	88.2	5.9	5.9
Dizziness	94.1	0	5.9

Table 3. Objective tumor response rates after external review

Best overall response	Patients (%)
Overall response rate	41.2
Complete response	5.9
Partial response	35.3
Stable disease	29.4
Progression	23.5
Not evaluable	5.9

Table 4. Prognostic factors

Outcome	Value
Median progression-free survival (months)	6.9
Median survival time (months)	25.1
One-year survival rate (%)	70.6

line treatment was 44.4% (4/9 patients: 4 with PR), i.e. a similar RR was achieved with second-line treatment ($P = 0.85$).

Complete response was achieved for a lung lesion, whereas PR was achieved for lymph node lesions in three patients, liver lesions in two patients, and both liver and lung lesions in one patient. No significant difference of response was noted among these sites ($P = 0.38$).

Survival

The median PFS was 6.9 months, the median survival time (MST) was 25.1 months and the 1-year survival rate was 70.6% (Table 4).

Responders had a median PFS of 15.0 months, MST of 43.6 months and 1-year survival rate of 100%, whereas the corresponding values for non-responders were 4 months, 10.6 months and 44.4%, respectively.

DISCUSSION

In the present study of TEGAFIRI, we employed the regimen that is widely used outside Japan. In this regimen, parenteral treatment is administered every 3 weeks in combination with 2 weeks of oral medication followed by a 1-week rest, and it is considered to be also applicable for use in Japan. Although the dose is set at 240–250 mg/m^2 for CPT-11 and 90 mg/day for LV when TEGAFIRI is given outside Japan (9,11,12), it was reduced to 150 mg/m^2 for CPT-11 and 75 mg/day for LV owing to restrictions imposed by the national health insurance scheme in Japan. For UFT, in contrast, the daily dose is 250 mg/m^2 outside Japan (9,11,12), whereas 300 mg/m^2/day (the standard domestic dosage) was used in the present study because the dose-limiting toxicity of diarrhea is less likely to occur in Orientals (5).

Although the dose of CPT-11 was lower in the present study than in overseas studies, the RR was similar in both cases. Polymorphism of the gene for UGT1A1, an enzyme participating in the metabolism of irinotecan, might lead to ethnic differences in the metabolism of this agent.

The incidence of Grade 3–4 adverse events showed lower tendency in responders than in non-responders ($P = 0.29$). This suggests that much efficacy cannot be expected in patients experiencing frequent adverse events.

In the present study, second-line treatment with TEGAFIRI achieved a similar effect to first-line treatment. Among the patients who received TEGAFIRI as second-line treatment, only one had received FOLFOX as first-line treatment and the others had been treated with 5-FU derivatives.

In the present study, the median PFS was 6.9 months and the MST was 25.1 months. These results are similar to the corresponding data reported for FOLFOX therapy (8.0 and 20.6 months) and for FOLFIRI therapy (8.5 and 21.5 months) (14). In the present study, the responders achieved a satisfactory outcome, with a median PFS of 15.0 months and an MST of 43.6 months. This outcome may have been achieved because the dose and regimen used in the present study were optimal, so that adverse events did not force patients to suspend treatment.

Now, the initial treatment for patients with advanced or recurrent CRC was FOLFIRI or FOLFOX in Japan. However, TEGAFIRI is one of the effective regimens for those who reject or cannot be performed continuous infusion of 5-FU or the operation of making central venous route. Further study on bevacizumab in combination with TEGAFIRI for patients with advanced or recurrent CRC is in preparation.

Dosages for Japanese patients should generally be determined on the basis of the results of Phase I trials conducted in Japan. For some drugs, however, we can also employ the large amounts of overseas data already obtained from more than one ethnic group. Therefore, it may be advisable to introduce overseas protocols for domestic clinical trials with the aid of overseas data, as was done in the present study.

In conclusion, the dose of CPT-11 approved in Japan is only 150 mg/m^2, but the RR obtained with TEGAFIRI using this dose was comparable to that obtained with full-dose TEGAFIRI outside Japan, and the responders achieved a good PFS of 15.0 months and an MST of 43.6 months.

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Conflict of interest statement

None declared.

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Original Paper

A New Classification System for Liver Metastases from Colorectal Cancer in Japanese Multicenter Analysis

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ABSTRACT

Background/Aims: Although numerous authors have reported various prognostic factors for liver metastases from colorectal cancer, there is not yet a general classification.

Methodology: A total of 478 colorectal cancer patients from 18 institutes were studied. Prognostic factors were investigated using univariate and multivariate analyses.

Results: Independent prognostic factors for colorectal liver metastases were number of liver metastases, size of the largest liver metastases, mesenteric lymph node metastases (pN0/1: ≤3 lesions, pN2: ≥4 lesions), and extrahepatic metastases (EM0: absence of extrahepatic metastasis, EM1: presence of extrahepatic metastases). We defined the following classification system; Stage A: HT1 (≤4 lesions and ≤5cm) and pN0/1, Stage B: HT2 (≥5 lesions or >5cm) and pN0/1, or HT1 and pN2, Stage C: HT2 and pN2, HT3 (≥5 lesions and >5cm) with any pN, or any HT and any pN with EM1. Five-year survival rates were 53.5% for Stage A patients, 25.4% for Stage B patients, and 5.8% for Stage C patients. Median survival time was 70.4 months, 31.4 months, and 17.2 months, respectively.

Conclusions: Our classification was advocated to evaluate prognoses for liver metastases from colorectal cancer. It can help guide decision making in terms of liver resection and assessing patient prognosis.

KEY WORDS:
Colorectal cancer; Liver metastasis; Classification system; Prognostic factor

INTRODUCTION

The incidence of colorectal cancer is ranked second among malignant diseases in Western countries and is the second leading cause of death (1). In Japan, more than 89,000 patients develop colorectal cancer and more than 36,000 die of this disease every year (2). The liver is the most common site of distant metastasis in colorectal cancer (3). It is well known that surgical resection is the most effective treatment for liver metastases from colorectal cancer (4-7). According to recent reports, 5-year survival after hepatectomy for colorectal cancer with liver metastases is 26% to 51% (4,8-16). However, some patients develop early recurrences and do not benefit from resection. It is important to stratify patients to determine which patients will most likely benefit from resection. Currently, we do not have any general rules for the treatment of colorectal cancer with liver metastases, and published papers are based on their own rule. The purpose of this study is to advocate a new classification system that could be used to help make treatment decisions for patients with liver metastases from colorectal cancer.

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METHODOLOGY

Patients

Patients with colorectal cancer with liver metastases registered in the "Study for Establishing Treatments for Hepatic and Pulmonary Metastases from Colorectal Cancer" were studied. The patients started treatment for liver metastases from colorectal cancer at 18 institutions from January 1992 to December 1996. This patient registry was established to investigate prognostic factors in colorectal cancer patients with liver metastases from a clinicopathologic viewpoint and to determine a classification system for patients. A total of 604 patients were enrolled for the study, and 478 patients were eligible for investigation. One hundred and twenty-six patients were not eligible because of incomplete data. The number of liver metastases, size of maximum liver metastases, lymph nodes metastases of the primary tumor, and extrahepatic distant metastases were evaluated for prognostic factors. For the resectable cases, the measurement of the size and the number of liver metastases was based on pathological findings. On the other hand, for the unresectable cases, computed

tomography was used to determine the size and number as well as extrahepatic disease.

The endpoint of this study was survival time. Death from any cause was considered an event. Patients who were still alive at last follow-up with or without disease were censored. Survival time was measured from the date of first resection of liver metastases to death or to the date of the last known follow-up evaluation in resectable cases, and was measured from the date of first diagnosis of liver metastases to death or to the date of the last known follow-up evaluation in unresectable cases.

TABLE 1 Characteristics of 478 Patients with Liver Metastases from Colorectal Cancer

Characteristics	Categories and Number / Average Value (Range)	
Patients		
Gender	Male	310
	Female	168
Age		60.5 (27-94)
Primary tumor	Colon	273
Location	Rectum	205
Tumor depth	T1	7
	T2	24
	T3	410
	T4	35
	Unknown	2
Lymph node	pN0	147
	pN1	219
	pN2	112
Liver	Synchronous	277
	Metachronous	201
Maximum diameter		4.0 (0.5-23.0)
Number of metastases		2.8 (1-22)
Extrahepatic metastases	Absence	392
	Presence	86

TABLE 2 Univariate Analysis of Prognostic Factors for Liver Metastases from Colorectal Cancer

Factors	5-year survival (%)	Median survival time (mo)	*P* Value
Over all	30.7	31.4	
Maximum diameter			
≤5cm	34.4	34.1	<0.0001
>5cm	16.8	26.4	
Number of liver metastases			
≤4 lesions	36.6	37.1	<0.0001
≥5 lesions	11.5	16.4	
Lymph node metastases			
≤3 lesions	36.8	36.2	<0.0001
≥4 lesions	13.1	21.5	
Extrahepatic metastases			
absence	36.2	35.4	<0.0001
presence	6.0	19.5	

TABLE 3 Univariate Analysis of Number of Liver Metastases

Number of Liver Metastases	Relative Risk	95% Confidence Interval Lower	Upper	*P* Value
1: ≥2	1.715	1.362	2.160	<0.0001
2≥: 3≥	2.066	1.653	2.577	<0.0001
3≥: 4≥	2.262	1.802	2.849	<0.0001
4≥: 5≥	2.326	1.818	2.967	<0.0001
5≥: 6≥	1.805	1.294	2.519	0.0005

Statistical Analysis

For patients with synchronous liver metastases, the survival period was calculated from the time of initial resection of the primary colorectal tumor. For patients with metachronous liver metastases, the survival period was calculated from the time of hepatectomy in resectable cases and was calculated from time of detection of liver metastases from colorectal cancer in unresectable cases. Survival curves were calculated using the Kaplan-Meier method. Statistical comparisons of potentially predictive factors were first performed using log-rank analysis for univariate analysis. The Cox proportional hazards model was used to perform multivariate analysis of factors related to survival. Significance was defined as $P<0.05$. All statistical evaluations were performed using Stat View® (Abacus Concepts, Inc., Berkley, California).

RESULTS

Characteristics

The clinicopathologic characteristics of eligible patients are summarized in **Table 1**. The lymph node metastases of primary tumor were pN0 in 147 patients, pN1 in 219 patients, and pN2 in 112 patients. The maximum diameter of metastatic tumors and number of liver metastases were 4.0cm (range, 0.5-23.0cm) and 2.8 (range, 1-22), respectively. Eighty-six patients had extrahepatic metastases, including hepatic hilar and para-aortic lymph node metastases; the remaining 392 patients did not have extrahepatic metastases. Of the 478 colorectal cancer patients with liver metastases, 380 cases were treated surgically. In this study, no patients were underwent radiofrequency ablation therapy.

Survival Analysis of Prognostic Factors

The 5-year survival rate was 30.7% and median survival time (MST) was 31.4 months **(Table 2)**. In our series, we examined the best point to draw the line: solitary *versus* multiple lesions, ≤2 lesions *versus* ≥3 lesions, ≤3 lesions *versus* ≥4 lesions, ≤4 lesions *versus* ≥5 lesions, ≤5 lesions *versus* ≥6 lesions **(Table 3)**. However, all of the comparisons resulted in significant statistical differences. Furthermore, ≤4 lesions *versus* ≥5 lesions had the highest relative risk (relative risk = 2.326). Therefore, the best point to draw the line was between ≤4 lesions and ≥5 lesions. Patients with ≤4 liver metastases lived significantly longer than patients with ≥5 lesions ($P<0.0001$). Survival at 5 years was 36.6% for ≤4 liver metastases patients and 11.5% for ≥5 liver metastases patients; MST was 37.1 months and 16.4 months, respectively.

We also examined the best point to draw the line in maximum size of liver metastases. When we categorized between ≤5cm and >5cm, we were able to obtain the highest relative risk and the lowest *P*

value **(Table 4)**. Patients with lesions ≤5cm lived significantly longer than patients with lesions >5cm (P<0.0001). Survival at 5 years was 34.4% in ≤5cm patients and 16.8% in >5cm patients; MST was 34.1 months and 26.4 months, respectively.

We categorized lymph nodes metastases of the primary tumor according to the tumor, node, and metastasis (TNM) classification. Patients in the pN0 and pN1 groups lived significantly longer than patients in the pN2 group (P<0.0001). Survival at 5 years was 36.8% in the pN0 and pN1 group and 13.1% in the pN2 group; MST was 36.2 months and 21.5 months, respectively. However, there were no significant differences between patients in the pN0 and pN1 groups. The 5-year survival was 33.0% in pN0 patients, 36.7% in pN1 patients, and 14.2% in pN2 patients; MST was 43.2 months, 31.3 months, and 21.9 months, respectively.

Patients with no extrahepatic metastases (EM0) lived significantly longer than patients with extrahepatic metastases (EM1) (P<0.0001). Survival at 5 years and MST, respectively, were 36.2% and 35.4 months for EM0 patients and 6.0% and 19.5 months for EM1 patients.

Multivariate Analysis and Classification for Liver Metastases from Colorectal Cancer

In the multivariate analysis, all of the prognostic factors were significantly different **(Table 5)**. Five-year survival rate was 40.1% in ≤4 lesions and ≤5cm liver metastases, 21.1% in ≤4 lesions and >5cm liver metastases, 14.2% in ≤5 lesions and ≤5cm liver metastases, and 5.2% in ≥5 lesions and >5cm liver metastases. Consequently, we defined ≤4 lesions and ≤5cm liver metastases as HT1; ≥5 lesions or >5cm liver metastases as HT2; and ≥5 lesions and >5cm liver metastases as HT3. As a result, patients with HT1 disease lived significantly longer than patients with HT2 disease (P<0.0001), who lived significantly longer than patients with HT3 disease (P<0.0001). Five-year survival rate was 39.2% in HT1 patients, 17.0% in HT2 patients, and 4.8% in HT3 patients. MST was 38.1 months, 26.0 months, and 12.0 months, respectively **(Figure 1)**.

Five-year survival rate was 48.1% in HT1 and pN0,1 patients, 22.5% in HT2 and pN0,1 patients, 7.7% in HT3 and pN0,1 patients, 18.9% in HT1 and pN2 patients, 3.1% in HT2 and pN2 patients, and 0.0% in HT3 and pN2 patients. Thus, we defined HT1 and pN0,1 as Stage A'; HT2 and pN0,1 or HT1 and pN2 as Stage B'; and HT2 and pN2 or HT3 with any pN as Stage C'. Stage A' patients lived significantly longer than Stage B' patients (P<0.0001), who lived significantly longer than Stage C' patients (P<0.0001). Five-year survival rate was 48.9% in Stage A' patients, 20.2% in Stage B' patients, and 4.0% in Stage C' patients. MST was 57.2 months, 27.4 months, and 14.7 months, respectively. Because patients with extrahepatic metastases showed poor prognosis, we defined that all of them were included in Stage C'. From the above classification, we were able to classify colorectal cancer patients with liver metastases; Stage A: HT1 and pN0/1, Stage B: HT2 and pN0/1, or HT1 and pN2, Stage C: HT2 and pN2, HT3 with any pN, or any HT and any pN with EM1

TABLE 4 Univariate Analysis of Size of Liver Metastases

Maximum Diameter of Liver Metastases	Relative Risk	95% Confidence Interval Lower	Upper	P Value
≤3cm: >3cm	1.324	1.056	1.661	0.0149
≤4cm: >4cm	1.524	1.212	1.916	0.0003
≤5cm: >5cm	1.623	1.279	2.137	<0.0001
≤6cm: >6cm	1.508	1.112	2.045	0.0081

TABLE 5 Multivariate Analysis of Prognostic Factors for Liver Metastases from Colorectal Cancer

Factors	Relative Risk	95% Confidence Interval Lower	Upper	P Value
Maximum diameter ≤5cm : >5cm	1.692	1.305	2.193	<0.0001
Number of liver metastases ≤4 : ≥5 lesions	2.326	1.818	2.967	<0.0001
Lymph node metastases ≤3 : >4 lesions	1.880	1.468	2.404	<0.0001
Extrahepatic metastases absence : presence	2.232	1.706	2.915	<0.0001

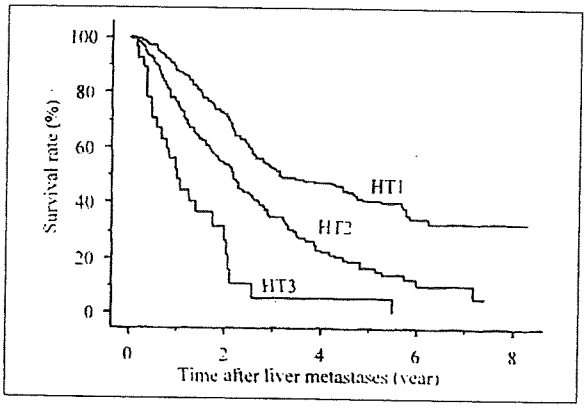

FIGURE 1 Survival curve after liver metastases according to HT factor. HT1: 4 lesions or less and 5cm or less, HT2: Except for HT1 and HT3, HT3: 5 lesions or more and more than 5cm.

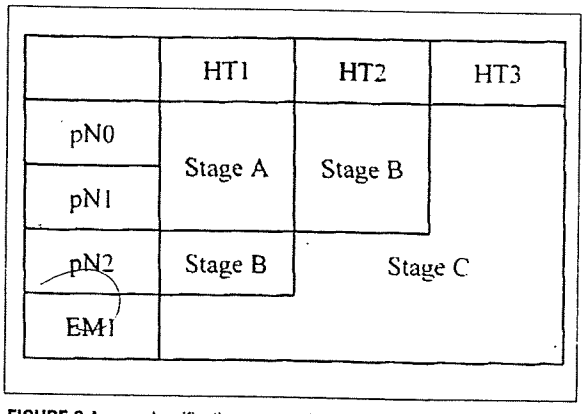

FIGURE 2 A new classification system for liver metastases from colorectal cancer. HT1, ≤4 lesions and ≤5cm; HT2, except for HT1 and HT3; ≥5 lesions and >5cm; EM1, presence of extrahepatic metastases.

(**Figure 2**). Five-year survival rate was 53.5% in Stage A patients, 25.4% in Stage B patients, and 5.8% in Stage C patients. MST was 70.4 months, 31.4 months, and 17.2 months, respectively (**Figure 3**).

Synchronous and Metachronous Liver Metastases

In synchronous cases, 5-year survival was 49.8% in Stage A patients, 25.9% in Stage B patients, and 4.6% in Stage C patients (**Table 6**). MST was 57.3 months, 31.5 months, and 17.1 months, respectively. In metachronous cases, 5-year survival was 57.1% in Stage A patients, 25.0% in Stage B patients, and 8.4% in Stage C patients. MST was 69.4 months, 28.6 months, and 17.9 months, respectively. Furthermore, in synchronous resectable cases, 5-year survival rate was 50.3% in Stage A patients, 33.9% in Stage B patients, and 8.2% in Stage C patients. In metachronous resectable cases, 5-year survival rate was 59.4% in Stage A patients, 28.4% in Stage B patients, and 9.9% in Stage C. In all subgroup, Stage A patients lived significantly longer than Stage B patients ($P<0.0001$), who lived significantly longer than Stage C patients ($P<0.0001$).

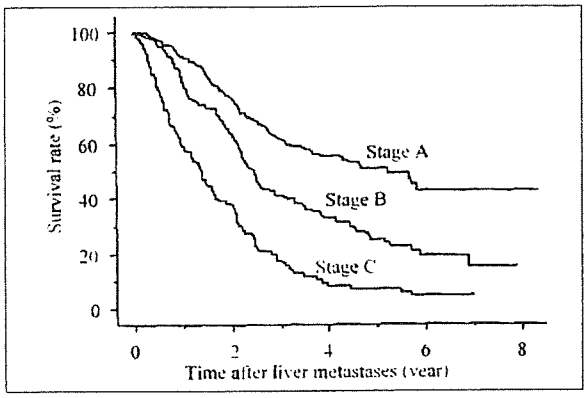

FIGURE 3 Survival curve after liver metastases according to a new classification of liver metastases from colorectal cancer.

TABLE 6 Long-term Survival after Liver Metastases based on Patients Staging

	5-year Survival (%)	Median Survival Time (months)	*P* Value
OVER ALL	30.7	31.4	
Stage A	53.5	70.4	<0.0001
Stage B	25.4	31.4	
Stage C	5.8	17.2	
Synchronous Cases	27.2	30.4	
Stage A	49.8	57.3	<0.0001
Stage B	25.9	31.5	
Stage C	4.6	17.1	
Metachronous Cases	35.7	35.4	
Stage A	57.1	69.4	<0.0001
Stage B	25.0	28.6	
Stage C	8.4	17.9	
Resectable Cases	36.8	38.5	
Stage A	54.9	70.4	<0.0001
Stage B	31.4	38.5	
Stage C	8.9	24.6	
Synchronous Cases	34.7	37.1	
Stage A	50.3	70.4	<0.0001
Stage B	33.9	38.5	
Stage C	8.2	24.8	
Metachronous Cases	39.4	40.5	
Stage A	59.4	-	<0.0001
Stage B	28.4	40.4	
Stage C	9.9	20.5	

DISCUSSION

The American Joint Committee on Cancer (AJCC) staging criteria categorize cases of colorectal cancer with liver metastases as stage IV (17). In Japan, a subclassification for liver metastases from colorectal cancer is commonly used (H0: no liver metastasis, H1: metastasis limited to one lobe, H2: some metastases in both lobes [≤4 lesions], H3: numerous metastases in both lobes [≤5 lesions]) (18). However, all patients with liver metastasis are classified as stage IV. The 5-year survival rate of patients with colorectal cancer with liver metastases ranges from 26% to 51%, yet no classification system is available for these patients. Clearly a need exists for a classification system for patients with colorectal cancer with liver metastases.

Many authors have reported prognostic factors for colorectal cancer liver metastases, including primary tumor stage (4,6,9,12-14,19), number of liver metastases (4,5,7-9,11-14,17,20), maximum size (4-6,7,9), carcinoembryonic antigen (CEA) level (4,5,7-9,16,20), time to liver metastases (5,9,13,14), and extrahepatic disease (7,8,12). Moreover, some investigators developed a scoring system for colorectal cancer liver metastases (4,8,10,14,20). However, none of these systems are available clinically, because of their complexity. During the last decade, more than 2500 articles on colorectal cancer with liver metastases have been published and appear on Medline. It is necessary to establish a common classification system to compare data across different studies.

Table 7 shows large studies for colorectal cancer with liver metastases. Hughes *et al.* listed prognostic factors, including positive mesenteric node in the primary tumor, the time to metastases, size of liver metastases >8cm, number of lesions >2, bilobar metastases, surgical margin >1cm, CEA level, and absence of chemotherapy (5). Nordlinger *et al.* proposed a prognostic scoring system based on seven factors: age older than 60 years, extension into serosa of the primary tumor, lymphatic spread of the primary tumor, size of the largest metastasis >5cm, disease-free interval >2 years, number of liver nodules >4, and resection margin >1cm (4). Scheele *et al.* proposed the following prognostic factors: the presence of satellite metastases, primary tumor grade, the time of metastasis diagnosis, diameter of the largest metastasis, anatomic *versus* nonanatomic approach, year of resection, and mesenteric lymph node involvement (9). Fong *et al.* attempted to score clinical risk

based on five factors: positive mesenteric lymph node in the primary tumor, disease-free interval >1 year, number of liver metastases >1, maximum size of liver metastases >5cm, and CEA level >200ng/mL (10). Although the more prognostic factors we incorporate into a classification system, the better the stratification will be, using too many variables will make the system too complicated. It is important that the classification is simple like the TNM classification system (17). The current classification system in this study incorporates four factors: lymph node metastases in the primary tumor, size of the largest liver metastasis, number of liver metastases, and extrahepatic disease, all of which are easy to remember. Moreover, it represents the prognosis of colorectal cancer liver metastases in synchronous and metachronous cases.

Past studies have reported that mesenteric lymph node metastases are one of the prognostic factors for colorectal cancer with liver metastases (4,9,10,13,14). Most authors investigated the presence or absence of primary lymph node metastases. We investigated the number of mesenteric lymph node metastases of the primary tumor. Although there was no significant difference between pN0 patients (no lymph node metastasis) and pN1 patients (1 to 3 metastases), pN2 patients (≤4 metastases) had a poor prognosis in terms of liver metastases. These findings suggested that pN0 and pN1 patients have an equivalent prognosis in terms of liver metastases. Thus, we drew the line between three and four lymph node metastases.

Though controversy exists (8,12,20), previous reports of large series have proposed the maximum diameter of the liver metastasis as a prognostic factor (4,5,9,10). From our experience, we agree that the largest liver metastasis is an independent prognostic factor. Moreover, we found the best point to draw the line at 5cm of maximum size.

The number of liver metastases has been reported by many authors to be a significant prognostic factor (4,5,6,8,10-12,14,19,20,21-24). Some have reported a significant difference between single and multiple lesions (6,10,12,21,22), and some have demonstrated poor prognosis for patients with ≥4 lesions (4,8,11,12,19,23,24). In our series, we examined the best point to draw the line. Our results indicated that the best point to draw the line was between ≤4 lesions and ≤5 lesions.

Extrahepatic disease has been demonstrated as a negative prognostic factor by many investigators (5,8,9,10). It is unlikely that patients with extrahepatic disease survive more than 5 years. However, according to Scheele *et al.*, curative resection of liver metastases with pulmonary metastases or local recurrence may prolong survival (25). However, in cases with other site recurrences, such as adrenal metastasis, omental deposit, nodules on the surface of the small bowel, and limited peritoneal spread, early recurrence has always resulted even though curative resection was accomplished. Beckurts *et al.* reported poor prognosis of hepatic hilum lymph node metastases. Hence, hilum lymph node metastases are included in extrahepatic disease (26).

TABLE 7 Past Published Large-sized Studies for Liver Metastases from Colorectal Cancer

	Patient number	Primary stage	Liver Size	Metastases Number	Extrahepatic metastases
Hughes, 1968	856	Y	Y	Y	Y
Nordlinger, 1995	1568	Y	Y	Y	-
Sheele, 1995	469	Y	Y	N	Y
Fong, 1999	1001	Y	Y	Y	Y
Current Study	478	Y	Y	Y	Y

Y, independent factor; N, not independent factor; -, not studied.

Although some patients have liver metastases at the time of diagnosis for primary colorectal cancer, others develop liver metastases metachronously. Our classification was suitable for either synchronous or metachronous liver metastases. To this date, no author has reported the classifications which are useful for both synchronous cases and metachronous cases.

This classification of liver metastases from colorectal cancer is not only simple but also useful for retrospective data in most institutes. Until now, there has been no classification system for liver metastases from colorectal cancer that can be used by all authors. Henceforth, we will be able to compare data from various studies and obtain new findings. Additionally, present study could be used to help make treatment decisions for patients with liver metastases from colorectal cancer.

Present classification was developed by retrospective data from limited institutions in Japan. Larger studies are necessary to prove the validity of our classification system.

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APPENDIX

The following institutions and investigators participated in the Study for Establishing Treatments for Hepatic and Pulmonary Metastases from Colorectal Cancer. They are listed in order of the number of cases recruited.

Aichi Cancer Center, Aichi: T Kato, Y Arai, M Suyama, H Nakanishi; Tokyo Metropolitan Komagome Hospital, Tokyo: T Mori, Y Nishimura; Tokyo Medical and Dental University, Tokyo: K Sugihara; National Defense Medical College, Saitama: H Mochizuki; National Cancer Center Hospital, Tokyo: J Yamamoto, H Kondo, T Akasu; Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka: M Higashiyama, M Kameyama; National Kyushu Cancer Center, Fukuoka: S Kohnoe; International Medical Center of Japan, Tokyo: Y Ishizaka; Nagoya

National Hospital, Aichi: M Kataoka; Osaka National Hospital, Osaka: Y Hasuike; Nara Medical University, Nara: S Nakajima; Tokyo Women's Medical University, Tokyo: S Kameoka; Kurume University, Fukuoka: Y Ogata; Kinki University, Osaka: K Okuno; Cancer Institute Hospital, Tokyo: S Okumura; Tokyo University, Tokyo: M Kawahara; Tokyo Metropolitan Bokuto Hospital, Tokyo: N Umekita; NTT West Osaka Hospital, Osaka: K Higashino; Hamamatsu University School of Medicine, Aichi: S Suzuki; Keio University, Tokyo: M Watanabe.

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A New Formula for Predicting Liver Metastasis in Patients with Colorectal Cancer: Immunohistochemical Analysis of a Large Series of 439 Surgically Resected Cases

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Key Words
Clinicopathological study · Colorectal cancer · Dysadherin · E-cadherin · Liver metastasis · Matrilysin

Abstract
Objective: The purpose of this study was to establish a new formula predicting liver metastasis in patients with colorectal cancer (CRC). ***Methods:*** Nine previously reported predictive markers for liver metastasis and/or prognosis (COX-2, dysadherin, E-cadherin, β-catenin, Ki-67, p53, laminin5γ2, matrilysin and MUC-1) were immunohistochemically investigated in 439 consecutive patients with CRC. We tried to determine the combination of molecules which best predicted liver metastasis. A formula for predicting liver metastasis was constructed using a training cohort comprising 150 cases, and applied to a validation cohort comprising 190 cases and another comprising 99 cases from an outside hospital. ***Results:*** A combination of dysadherin, E-cadherin and matrilysin was identified to be best for predicting liver metastasis (area under the curve value, 0.807). The predictive formula: 3× dysadherin score [0 for low expression (≤50% of tumor cells positive) or 1 for high expression (>50%)] + 4× E-cadherin score [0 for preserved (>80% of tumor cells positive) or 1 for reduced (≤80%)] + 2× matrilysin score [0 for low expression (≤30% of tumor cells positive) or 1 for high expression (>30%)] was able to discriminate patients with liver metastasis in the training cohort with a sensitivity of 85.7% and a specificity of 58.9%. The discriminative capacity of the formula was validated in the first cohort with a sensitivity of 87.0% and a specificity of 66.5%, and in the second cohort with a sensitivity of 80% and a specificity of 60.0%. ***Conclusions:*** We have established a formula for predicting liver metastasis in patients with CRC, and confirmed that it has a high sensitivity potentially useful for clinical application.

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Introduction

Colorectal cancer (CRC) is the third most common malignant tumor in the world [1]. Its prognosis after curative resection depends exclusively on the development of metachronous metastases, especially liver metastasis [1]. To improve the prognosis of CRC, the most important considerations are the selection of patients at high risk for liver metastasis and subsequently the institution of appropriate adjuvant therapy. Adjuvant therapy in patients with CRC after curative resection has been reported to be useful for improving overall and disease-free survival [2–4]. Resection of liver metastases offers a chance for prolonged survival [5, 6]. Patients with intermediate-stage disease (stage II or III) have a recurrence rate of about 20–50%, including liver and lung metastases, recurrence in lymph nodes and peritoneal dissemination [2, 3, 7]. The remaining 50–80% have no recurrence, and therefore these patients underwent unnecessary adjuvant chemotherapy. To increase the survival benefit from adjuvant chemotherapy and the early detection rate of surgically resectable liver metastasis, the selection of patients at high risk for liver metastasis is essential.

Conventional risk factors for liver metastasis include lymph node metastasis, venous, serosal and lymphatic invasion, tumor dedifferentiation, white streak sign and resection margin [1, 8–14]. The accuracy of diagnosing liver metastasis using these conventional markers has been reported to be between 24 and 98% in terms of sensitivity, and between 34 and 97% in terms of specificity [1, 8–13]. Recently, many molecular markers have been reported to be useful for predicting liver metastasis and thus prognosis in CRC patients [15–19]. Therefore, in the present study, we tried to determine the best combination of the immunohistochemically detectable molecules already reported for predicting liver metastasis, and to establish a new formula for accurate prediction of liver metastasis in CRC patients.

Materials and Methods

Patients and Samples

Four hundred thirty-nine patients with CRC were selected from the lists of patients treated at the National Cancer Center Hospital (Tokyo, Japan) between 1995 and 1998 and the Kitasato University (Kanagawa, Japan) between 2000 and 2002. The patients included 267 (60.8%) men and 172 (39.2%) women, ranging in age from 21 to 93 years (median 62 years). Sample selection was restricted to consecutive cases diagnosed as stage II (44.2%, 194 of 439) or III (55.8%, 245 of 439). All patients had undergone curative resection. None of the patients had received chemotherapy or radiotherapy preoperatively. Follow-up studies were complete in all patients, ranging from 0.1 to 8.3 years (median, 5.5 years). Two patients who were followed up for 0.1 months died of pulmonary embolism 3 and 4 days after surgery, respectively. Recurrence after surgery was diagnosed by ultrasonography, computed tomography and angiography. Tumor location, lymph node, liver and lung metastases, tumor size, and lymphatic and venous invasion were all classified according to the TNM classification [20]. Histologically, tumors were classified according to the International Histological Classification of Tumors of the World Health Organization [21]. Among the study cases, 188 (42.8%) were classified as well-differentiated adenocarcinomas, 231 (52.6%) as moderately differentiated, 11 (2.5%) as poorly differentiated, 6 (1.37%) as mucinous and 2 (0.46%) as signet-ring-cell adenocarcinomas. During the follow-up period, liver metastases were observed in 49 (11.2%) cases, and at the time of writing this has proved fatal in 28 (57.2%) cases.

We divided the 439 patients into three groups. Group I included 150 consecutive patients, 94 men (62.7%) and 56 women (37.3%), ranging in age from 21 to 87 years (median, 63 years), operated on at the National Cancer Center Hospital between January 1, 1995, and July 1, 1996. In group I, 21 patients (14%) developed liver metastases and were used as a training cohort. Group II included 190 consecutive patients, 116 men (61.1%) and 74 women (38.9%), ranging in age from 32 to 93 years (median, 62 years), who were operated on at the National Cancer Center Hospital between July 1, 1996, and January 1, 1998. In group II, 24 patients (12.6%) developed liver metastases; they were used as the first validation cohort. Group III included 99 consecutive patients, 57 men (57.6%) and 42 women (42.4%), ranging in age from 27 to 85 years (median, 62 years), who were operated on at the Kitasato University between January 1, 2000, and January 1, 2003. In group III, 5 patients (5.1%) developed liver metastases; they were used as the second validation cohort.

Search Strategy and Selection Criteria for Antibodies

We selected nine previously reported molecules for immunohistochemical study – β-catenin [22–26], cyclooxygenase-2 (COX-2) [16, 27, 28], dysadherin [18, 29–31], E-cadherin [18, 23, 32], Ki-67 [33, 34], p53 [11, 34–36], matrilysin [37, 38], MUC-1 [19, 33] and laminin5γ2 [17, 39, 40] – as the prognostic significance of the expression of these markers has already been reported in several papers in which multivariate logistic regression analysis was performed, and reliable figures and descriptions of immunostaining were demonstrated (table 1).

Immunohistochemistry

Resected primary colon cancers were cross-sectioned in order to obtain tissue sections according to the general rules for clinical and pathological studies on cancer of the colon, rectum and anus [41]. Representative tissue sections taken at the maximum cross-section, each containing the deepest site of cancer invasion, were subjected to immunohistochemical staining using the avidin-biotin peroxidase complex method [42]. After deparaffinization in xylene and rehydration in ethanol, the sections were heated in citrate buffer (10 mM, pH 6.0) at 120°C for 10 min for antigen retrieval. Endogenous peroxidase was blocked with 0.3% hydrogen peroxidase in methanol for 20 min. The sections were then incubated with anti-dysadherin antibody (M53; 1:500 dilution, established in our laboratory [31]), anti-E-cadherin antibody (HECD-

Table 1. List of antibodies used and working conditions

Antibody	Clone	Dilution	AR	City/location	Source
β-Catenin	14	1:5,000	MW	Lexington/Ky./USA	Transduction
COX-2	160112	1:200	MW	Ann Arbor/Mich./USA	Cayman
Dysadherin	M53	1:4,000	MW	Tokyo/Japan	original
E-cadherin	HECD-1	1:4,000	MW	Tokyo/Japan	original
Ki-67	MIB-1	1:500	MW	Glostrup/Denmark	DAKO
Laminin5γ2	1-97	1:4,000	MW	Tokyo/Japan	original
Matrilysin	141B-2	1:800	MW	Tokyo/Japan	Fine Chemical
MUC-1	Ma695	1:200	MW	Newcastle/UK	Novocastra
p53	DO-7	1:500	MW	Newcastle/UK	Novocastra

AR = Antigen retrieval; MW = microwave.

1; 1:2,000 dilution, established in our laboratory [43]), anti-β-catenin antibody (clone 14; 1:5,000 dilution, Transduction Laboratories, Lexington, Ky., USA), anti-COX-2 antibody (160112; 1:200 dilution, Cayman, Ann Arbor, Mich., USA), anti-laminin5γ2 antibody (1–97; 1:4,000 dilution, established in our laboratory [40]), anti-Ki-67 antibody (MIB-1; 1:500 dilution, Dako, Glostrup, Denmark), anti-matrilysin antibody (141B-2; 1:800 dilution, DFC, Toyama, Japan), anti-MUC-1 antibody (Ma695; 1:200 dilution, Novocastra, Newcastle-upon-Tyne, UK) and anti-p53 antibody (DO7; 1:500 dilution, Novocastra) at 4°C. The sections were washed with phosphate-buffered saline, incubated with biotin-labeled anti-mouse IgG antibody and avidin-biotin complex (ABC kit, Vector Laboratories, Peterborough, UK) and visualized using diaminobenzidine tetrahydrochloride. The sections were counterstained with hematoxylin. As internal positive controls for dysadherin and laminin5γ2 staining, positive staining of endothelial cells present in the primary tumor tissue was used. As an internal positive control for E-cadherin staining, membranous staining of normal epithelial cells adjacent to the tumor specimens was used. As internal positive controls for COX-2, MUC-1, β-catenin, matrilysin, p53 and Ki-67 staining, colon cancer samples known to stain positively for each antibody were used. As a negative control, normal mouse IgG (Vector Laboratories, Burlingame, Calif., USA) was used instead of the primary antibody.

Evaluation of Immunohistochemistry

All the slides were first reviewed by two observers (H.O. and Y.N.) independently without knowledge of the clinical data. All discrepancies were resolved by joint review of the slides in question. After selecting three markers – dysadherin, E-cadherin and matrilysin – from the training cohort, group I, immunohistochemical stainings were scored by a third independent pathologist (Y.F.) to allow validation of the evaluation of the immunohistochemical results.

The percentages of tumor cells positive for p53, Ki-67, β-catenin, COX-2, laminin5γ2, dysadherin, E-cadherin and MUC-1 were evaluated semiquantitatively as the ratio of the number of positive tumor cells relative to the total number of tumor cells. Cutoff indices were fixed according to previous reports as follows. Expression of E-cadherin was defined as preserved when membrane staining of >80% of the tumor cells was observed and reduced when membrane staining ≤80% of the tumor cells was observed [18]. Expression of dysadherin and β-catenin was defined as high when membrane staining >50% of the tumor cells was observed, and as low when membrane staining ≤50% of the cells was observed [18]. Expression of laminin5γ2 was categorized into three groups as: few, <10% of tumor cells positive; moderate, 10–50% of tumor cells positive, and high, >50% of tumor cells positive [17]. Expression of matrilysin was defined as high when >30% of tumor cells were stained at the invasive front, and as low when ≤30% of cells were stained at the invasive front [15, 38]. Expression of COX-2 was defined as positive when cytoplasmic staining of >10% of tumor cells was observed [16]. Expression of MUC-1 [19] and p53 and Ki-67 [34] was defined as positive when >10% of tumor cells were stained.

Statistical Analysis

All the data were tabulated, and statistical tests were performed with SAS version 9.1 (SAS Institute, Cary, N.C., USA). The relationship between clinicopathological findings and the scores of immunohistochemical markers were analyzed by Fisher's exact test for a two-by-two contingency table or by the χ^2 test for other contingency tables.

Selection of the best combination of markers was performed in group I by a stepwise selection procedure in a multivariate logistic regression model. The stepwise procedure was set to a threshold of 0.05 for inclusion and 0.15 for exclusion. Each selected independent liver metastasis factor was given a coefficient suggested by the multivariate logistic regression model, as a parameter estimate. In order to evaluate the goodness of fit for the final model, we applied the Hosmer-Lemeshow test [44] on eight distinct groups, and the Akaike Information Criterion (AIC) test [45] to the combination set of markers. AIC is widely used as a criterion for model selection. The model with the minimum AIC is chosen as the best one, and the AIC is therefore formally biased against overly complex models. The immunohistochemical metastatic score (IMS) was calculated according to the formula composed of selected factors. The scoring formula was applied to patients in groups II and III as well as those in group I. The thresh-

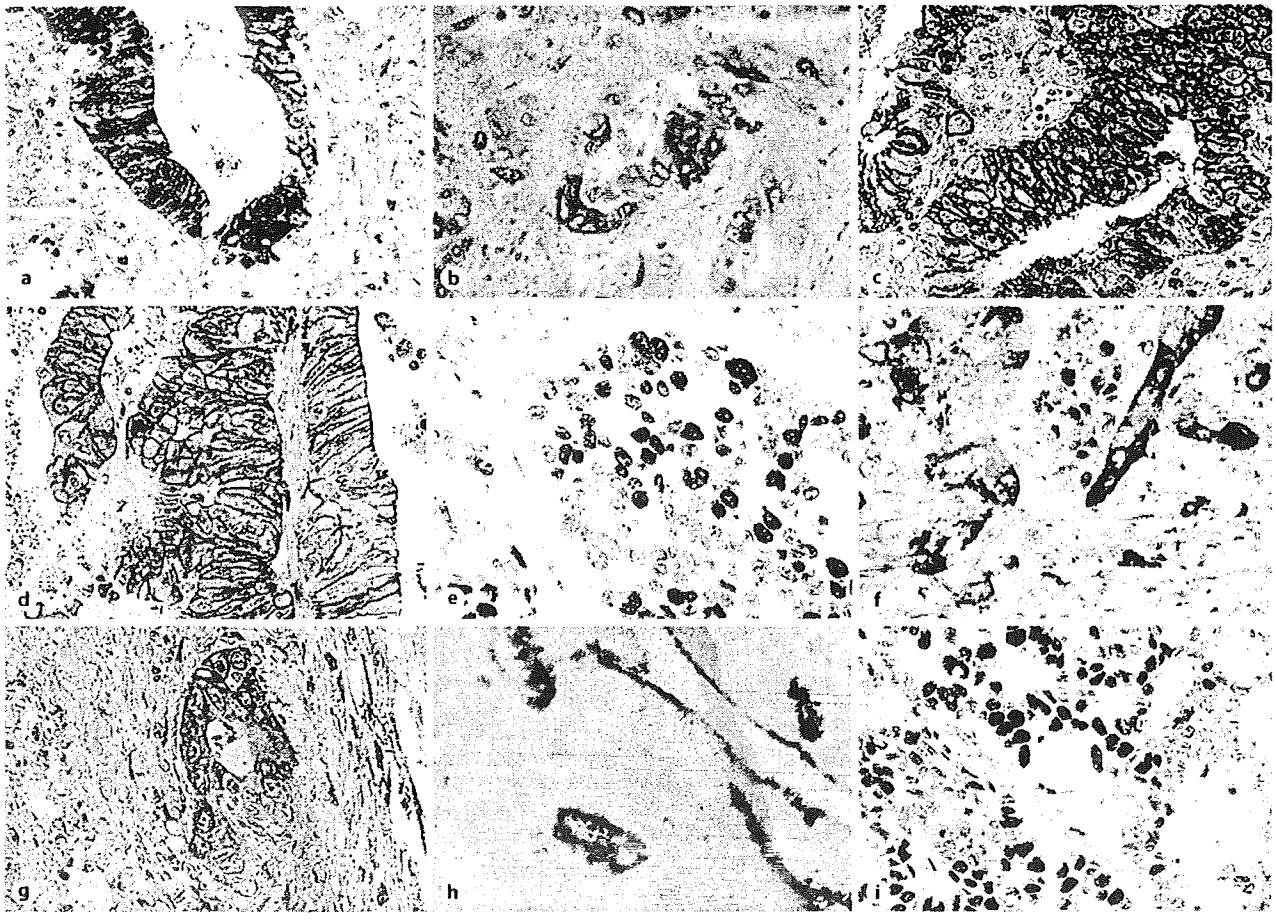

Fig. 1. Immunohistochemical staining pattern of each molecular marker (×400). β-Catenin expression was localized at the cell-cell borders, in the cytoplasm and in the nuclei of cancer cells (**a**). COX-2 expression was observed in the cytoplasm of cancer cells (**b**). Membranous dysadherin (**c**) and E-cadherin (**d**) expression was observed at the cell-cell borders of cancer cells. Ki-67 (**e**) and p53 expression (**i**) was observed in the nuclei of cancer cells. Laminin5γ2 (**f**) and matrilysin expression (**g**) was predominately intracytoplasmic, and preferentially located at the invasive front. MUC-1 (**h**) expression was located at the surface of glandular structures of cancer cells.

old was set at five points. Two theoretical potential groups at risk for liver metastasis were defined as follows: group A, low risk of liver metastasis, total score 0 ≤ IMS ≤ 4; group B, high risk of liver metastasis, total score 5 ≤ IMS.

Results

Biomarkers in Primary Colon Cancers with Respect to the Occurrence of Liver Metastasis

The associations between clinicopathological factors and liver metastasis in all samples are shown in table 2. The representative staining pattern of each molecular marker is shown in figure 1. The associations between liver metastasis and immunohistochemical molecular markers in group I are shown in table 3. There was a significant association between liver metastasis and E-cadherin (p = 0.001), laminin5γ2 (p = 0.005), dysadherin (p = 0.004) and matrilysin expression (p = 0.017; table 3).

Identification of Candidate Markers in the Training Cohort, Group I, by Stepwise Analysis of the Logistic Regression Model

Although two markers – dysadherin and E-cadherin – were significantly associated with liver metastasis (p = 0.013 and 0.004, respectively) by the multivariate re-