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ORIGINAL ARTICLE

Clinical usefulness of *KRAS*, *BRAF*, and *PIK3CA* mutations as predictive markers of cetuximab efficacy in irinotecan- and oxaliplatin-refractory Japanese patients with metastatic colorectal cancer

Hiroshi Soeda · Hideki Shimodaira · Mika Watanabe · Takao Suzuki · Makio Gamoh · Takahiro Mori · Keigo Komine · Noriyuki Iwama · Shunsuke Kato · Chikashi Ishioka

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Abstract
Background Anti-epidermal growth factor receptor (EGFR) antibodies, cetuximab, and panitumumab are established as a new treatment option for metastatic colorectal cancer (mCRC). Among activating mutations downstream of EGFR, the *KRAS* mutation, which is present in 30–45 % of CRC patients, has shown to be a predictive biomarker of resistance to anti-EGFR antibody therapy based on Caucasian studies.
Methods Forty-three chemotherapy-refractory Japanese patients with mCRC were treated with cetuximab monotherapy or cetuximab plus irinotecan. *KRAS*, *BRAF*, and *PIK3CA* mutational status of tumors was assessed. The association between mutational status and treatment outcome was evaluated.
Results Of 43 tumors, *KRAS*, *BRAF*, and *PIK3CA* mutations were identified in 12 (27.9 %), 2 (4.7 %), and 2 (4.7 %) tumors, respectively. The wild-type *KRAS* subgroup showed better clinical outcomes than the mutant *KRAS* subgroup in terms of response rate (RR) (31.3 % vs. 0 %, $P = 0.034$) and progression-free survival (PFS) (5.1 vs. 3.0 months, $P = 0.017$). No responder to treatment was shown in 16 (37.2 %) patients with tumors harboring mutations in any one of the three genes (*KRAS*, *BRAF*, and *PIK3CA*). The wild-type subgroup without any mutations in *KRAS*, *BRAF*, and *PIK3CA* had a better RR (37.0 %) and PFS (6.4 months) than did the wild-type *KRAS* subgroup.
Conclusion Our data indicated that *KRAS* status is predictive of cetuximab response in the Japanese population. The additional analysis of *BRAF* and *PIK3CA* genes in wild-type *KRAS* patients could improve selection of patients who are most likely to benefit from anti-EGFR antibody therapy.

Keywords Cetuximab · Colorectal cancer · *KRAS* · *BRAF* · *PIK3CA*

H. Soeda · H. Shimodaira · K. Komine · S. Kato · C. Ishioka (✉)
Department of Clinical Oncology, Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryo-machi, Aobaku, Sendai 980-8575, Japan
e-mail: chikashi@idac.tohoku.ac.jp

H. Shimodaira · S. Kato · C. Ishioka
Department of Clinical Oncology, Tohoku University Hospital, Sendai, Japan

M. Watanabe
Department of Pathology, Tohoku University Hospital, Sendai, Japan

T. Suzuki
Department of Medical Oncology, Sendai Medical Center, Sendai, Japan

M. Gamoh
Department of Clinical Oncology, South Miyagi Medical Center, Ogawara, Japan

T. Mori · C. Ishioka
Cancer Center, Tohoku University Hospital, Sendai, Japan

N. Iwama
Department of Pathology, Sendai Kousei Hospital, Sendai, Japan

Introduction

Epidermal growth factor receptor (EGFR), a receptor tyrosine kinase, triggers a downstream signaling cascade through such as the RAS–RAF–MAPK and PI3K–AKT pathways, which are involved in cell proliferation, survival, and motility. Inhibition of EGFR activation has

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demonstrated significant promise as a molecular targeting therapy for various solid tumors. Two monoclonal antibodies (mAbs) targeting EGFR, cetuximab and panitumumab, have been approved for treatment of metastatic colorectal cancer (mCRC). The initial candidate biomarker for the anti-EGFR antibody response, EGFR expression analyzed by immunohistochemistry, was not a reliable predictive factor [1]. *KRAS*, downstream of EGFR, was shown to be a useful biomarker because somatic mutations that mainly occur in codons 12 and 13 result in constitutive activation of the RAS–MAP pathway regardless of EGFR inhibition [2–4]. A number of groups undertook retrospective *KRAS* testing of tumors from mCRC patients who were treated with cetuximab or panitumumab [5, 6]. Studies of patients receiving first and subsequent lines of treatment have found that those with mutated *KRAS* do not respond to, or experience any survival benefit from, treatment with anti-EGFR mAb [2–4, 6–10]. However, only a small proportion of patients achieved an objective response and benefit from cetuximab even among those with wild-type *KRAS* tumors. Thus, other downstream factors in EGFR signaling are now being explored, such as *BRAF* and *PIK3CA*, which are mutated in 5–10 % and 10–30 % of CRC, respectively.

Activating mutations in *BRAF*, which is mutually exclusive with *KRAS* mutations, may be responsible for the lack of efficacy of anti-EGFR mAbs in wild-type *KRAS* tumors [11, 12]. Retrospective analyses of anti-EGFR mAb-based treatment in various lines showed a correlation between the *BRAF* V600E and resistance to anti-EGFR mAb [11, 13]. *BRAF* mutation also has been shown to be both a prognostic factor and predictive of cetuximab response [13]. Therefore, interpretation of the clinical significance of *BRAF* mutations is complicated. The *PIK3CA* gene encodes the catalytic subunit p110α of PI3K. Tumor-derived mutant PI3K stimulates the AKT pathway and promotes cell growth in several cancers, including CRC. Tumors with *PIK3CA* mutations are associated with poor prognosis. Mutations in the *PIK3CA* gene have been shown to significantly impair response to treatment with anti-EGFR mAbs in mCRC patients. However, recent contradictory evidence indicates no strong rationale for using *PIK3CA* mutations as a single predictive marker for cetuximab response in chemotherapy-refractory mCRC [14]. A large-scale European study reported that the combination of *KRAS*, *BRAF*, *NRAS*, and *PIK3CA* mutation status improved prediction sensitivity for anti-EGFR mAb response [15].

The epidermal growth factor receptor is a critical predictive marker of gefitinib efficacy in non-small cell lung cancer (NSCLC). A clear ethnic difference in the frequency of EGFR mutations was found between Caucasians and Asians. The mutation frequency is higher in Asian NSCLC patients (about 30–60 %) than in Caucasian patients (approximately 10–20 %) [16–18]. However, the ethnic differences between Caucasians and Asians in mutation prevalence of *KRAS*, *BRAF*, and *PIK3CA* in mCRC have not been evaluated fully. Moreover, *KRAS* mutation status and that of other EGFR-downstream genes should be validated as predictive markers of anti-EGFR therapy in the Asian population.

We evaluated the relationship between *KRAS* mutation status and response to cetuximab-based treatment in Japanese patients with mCRC who have failed prior chemotherapy including irinotecan, oxaliplatin, and fluoropyrimidine. Furthermore, to optimize the selection of patients who are most likely to benefit from anti-EGFR mAbs, we investigated the association of minor *KRAS* mutations in codon 61, *BRAF* V600E mutation, and *PIK3CA* mutations in exons 9 and 20 with clinical outcomes.

Materials and methods

Patients and trial design

This study, aimed to examine the effect of cetuximab on RR and PFS among patients with mCRC in whom all prior chemotherapy had failed and for whom no other standard anticancer therapy was available, was approved by the Ethical Committee of Tohoku University School of Medicine. Eligible patients were enrolled between October 2008 and May 2010. Tumor specimens of all patients exhibited EGFR expression in >1 % of malignant cells, as determined by immunohistochemistry with the Dako EGFR PharmDx kit (DakoCytomation, Glostrup). None of the patients had received previous treatment with anti-EGFR mAb. After enrollment, patients received cetuximab-based treatment. Cetuximab was administered intravenously at a standard dosage of 400 mg/m^2 over 2 h on day 1 of treatment, followed by 250 mg/m^2 intravenously over 1 h, once a week. Irinotecan was administered intravenously at a standard dosage of 150 mg/m^2 every 2 weeks or 100 mg/m^2 weekly for 3 consecutive weeks, following by a 1-week rest. Patients were evaluated for tumor response or progression every 8 weeks by radiologic imaging. Cetuximab-based treatment was continued until disease progression or unacceptable toxicity occurred.

Tumor collection and processing

Formalin-fixed, paraffin-embedded (FFPE) samples of tumor tissue from archival specimens collected at the time of diagnosis were stored at Tohoku University Hospital. Assays of tissue samples for *KRAS*, *BRAF*, and *PIK3CA*

mutations were performed at the Department of Clinical Oncology, Institute of Development, Aging and Cancer, Tohoku University. All patients' samples were screened for *KRAS* mutation in codons 12, 13, and 61, and for *BRAF* V600E and *PIK3CA* mutations in exons 9 and 20. All available tissue samples were classified as mutant or wild type.

Nucleotide sequence analysis

Mutation analyses of *KRAS*, *BRAF*, and *PIK3CA* were performed by extraction of genomic DNA from FFPE tissue slides or sections. DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen) according to the manufacturer's protocol. Analyses of the DNA sequences were performed with the use of the automated CEQ2000XL DNA analysis system (Beckman Coulter) under specific cycle and temperature conditions. The PCR products were analyzed by 1.0 % agarose gel electrophoresis. Appropriate positive and negative controls were included for *KRAS*, *BRAF*, and *PIK3CA*. To minimize bias, the persons who performed the mutation analyses were blinded to clinical outcomes.

Statistical analysis

All patients for whom data on *KRAS*, *BRAF*, and *PIK3CA* mutation status were available were included in the analysis. The statistical analyses of categorical variables were performed using the χ^2 test. RR was defined according to the Response Evaluation Criteria in Solid Tumors (RECIST) ver. 1.0. According to RECIST criteria, patients were categorized as responders if they achieved complete response (CR) or partial response (PR), or nonresponders if they showed stable disease (SD) or progressive disease (PD). PFS was defined as the time from the beginning of chemotherapy until the first objective evidence of disease progression or death from any cause. The PFS analyses were determined according to the Kaplan–Meier method, and survival curves were compared using the log-rank test. Statistical significance was set at $P < 0.05$ for a bilateral test.

Table 1 Patient characteristics

	All	*KRAS* mutant	*KRAS* wild type
Total number of patients	43	12	31
Median age, years (range)	57 (31–80)	56 (41–80)	63 (31–79)
Gender			
Male	25	6	19
Female	18	6	12
ECOG performance status			
0	29	10	19
1	13	2	11
2	1	0	1
Number of previous chemotherapy lines			
1	0	0	0
2	25	8	17
≥3	18	4	14
Prior chemotherapy for advanced disease			
FOLFOX	43	12	31
FOLFIRI/IRIS/Irinotecan/IFL	33/5/3/2	10/1/0/1	23/4/3/1
Bevacizumab	17	4	13
Chemotherapy regimen			
Cetuximab + irinotecan	31	12	19
Cetuximab alone	12	0	12
Primary tumor			
Cecum	2	1	1
Ascending colon	8	3	5
Transverse colon	3	1	2
Descending colon	0	0	0
Sigmoid colon	12	2	10
Rectum	18	5	13
Metastatic sites			
Liver	32	9	23
Lung	27	8	19
Intraabdominal lymph nodes	15	2	13
Peritoneum	7	2	5
Bone	3	0	3
Others	6	2	4

FOLFOX 5-fluorouracil, leucovorin, oxaliplatin, *FOLFIRI* 5-fluorouracil, leucovorin, irinotecan, *IRIS* irinotecan, S-1, *IFL* irinotecan, 5-fluorouracil, leucovorin

Results

Patient characteristics

Patient clinical characteristics are listed in Table 1: 43 patients received cetuximab-based treatment. Of these, 42 patients were ECOG performance status 0 or 1, and only 1 patient was ECOG performance status 2.

All patients had failed prior chemotherapy including irinotecan, oxaliplatin, and fluoropyrimidine. None of the patients had been treated with anti-EGFR mAbs. Prior oxaliplatin-containing regimen included only the FOLFOX regimen [infusion and bolus 5-fluorouracil (5-FU) plus oxaliplatin]. Prior irinotecan-containing therapies included the FOLFIRI regimen (infusion and bolus 5-FU with irinotecan) in 33 patients, irinotecan monotherapy in 3

patients, S-1 plus irinotecan in 5 patients, and the IFL regimen (bolus 5-FU plus irinotecan) in 2 patients. Seventeen patients received bevacizumab in their treatment regimen.

The sites of metastases were liver (32; 74.4 %), followed by lung (27; 62.8 %), intraabdominal lymph nodes (15; 34.9 %), and peritoneum (7; 16.3 %). Among 43 patients with mCRC, 31 (72.1 %) received cetuximab plus irinotecan and 12 (27.9 %) received cetuximab monotherapy.

Toxicity

Toxicity data are summarized in Table 2. Grade 3–4 neutropenia was observed in 12 patients (27.9 %), and grade 3–4 anemia was observed in 4 (9.3 %). Skin toxicity, including acne, rash, dry skin, pruritus, acneiform dermatitis, and papular rash, was observed in 42 (97.7 %) patients. Grade 3–4 skin toxicity was observed in 4 patients (9.3 %). Other grade 3–4 toxicities included diarrhea (2.3 %), stomatitis (2.3 %) and hypomagnesia (2.3 %). The toxicity profiles did not differ between patients with wild-type *KRAS* tumors and those with mutated *KRAS* tumors.

Mutation analyses of *KRAS*, *BRAF*, and *PIK3CA*

Table 3 provides a list of mutations detected by direct sequencing. We analyzed a relatively rare mutation in codon 61 in addition to the common mutations in codons 12 and 13 to increase the sensitivity of mutation detection. *KRAS* mutations at codons 12, 13, and 61 were observed in 12 (27.9 %) of the tumors. Of the 11 detected mutations in codons 12 and 13, the most frequent mutation was G12D (14.0 %), followed by G13D (7.0 %), G12V (2.3 %), and G12A (2.3 %). Q61H was found in 1 tumor (2.3 %). Two of the three common *KRAS* mutations, G12D, G13D, and G12V, were also detected frequently in this study. *BRAF* mutation at codon 600 (V600E) was observed in 2 tumors (4.7 %), both of which were *KRAS* wild type. *PIK3CA* mutations in exon 9 (E542K and E545G) were observed in 2 patients (4.7 %), but no tumor mutations were found in exon 20.

Table 2 Toxicity profile in 43 mCRC patients

Event	All ($n = 43$)		*KRAS* mutant ($n = 12$)		*KRAS* wild type ($n = 31$)	
	G1–4 (%)	G3–4 (%)	G1–4 (%)	G3–4 (%)	G1–4 (%)	G3–4 (%)
Leukopenia	16 (37.2)	5 (11.6)	4 (33.3)	2 (16.7)	12 (38.7)	3 (9.7)
Neutropenia	18 (41.9)	12 (27.9)	4 (33.3)	4 (33.3)	14 (45.2)	8 (25.8)
Anemia	11 (25.6)	4 (9.3)	1 (8.3)	0 (0)	10 (32.3)	4 (12.9)
Thrombocytopenia	2 (4.7)	0 (0)	1 (8.3)	0 (0)	1 (3.2)	0 (0)
Diarrhea	11 (25.6)	1 (2.3)	1 (8.3)	0 (0)	10 (32.3)	1 (3.2)
Skin toxicity	42 (97.7)	4 (9.3)	12 (100)	1 (8.3)	30 (96.8)	3 (9.7)
HFS	8 (18.6)	0 (0)	1 (8.3)	0 (0)	7 (22.6)	0 (0)
Stomatitis	15 (34.9)	1 (2.3)	4 (33.3)	0 (0)	11 (35.5)	1 (3.2)
Nausea	12 (27.9)	0 (0)	2 (16.7)	0 (0)	10 (32.3)	0 (0)
Vomiting	5 (11.6)	0 (0)	0 (0)	0 (0)	5 (16.1)	0 (0)
Fatigue	16 (37.2)	0 (0)	3 (25.0)	0 (0)	13 (41.9)	0 (0)
Anorexia	10 (23.3)	0 (0)	2 (16.7)	0 (0)	8 (25.8)	0 (0)
Hypomagnesia	11 (25.6)	1 (2.3)	1 (8.3)	0 (0)	10 (32.3)	1 (3.2)

HFS hand–foot syndrome

Table 3 *KRAS*, *BRAF*, and *PIK3CA* mutation frequencies ($n = 43$)

Gene	Codon	Nucleotide substitution	Amino acid substitution	Number (%)	
KRAS	12	GGT → GAT	G12D	6 (14.0)	12 (27.9)
		GGT → GCT	G12A	1 (2.3)	
		GGT → GTT	G12V	1 (2.3)	
	13	GGC → GAC	G13D	3 (7.0)	
	61	CAA → CAC	Q61H	1 (2.3)	
BRAF	600	GTG → GAG	V600E	2 (4.7)	2 (4.7)
PIK3CA	542	GAA → AAA	E542K	1 (2.3)	2 (4.7)
	545	GAG → GGG	E545G	1 (2.3)	

Table 4 Response to cetuximab according to the presence or absence of gene mutations in the 43 patients

Tumor response	*KRAS* status in codons 12, 13		Genetic status of *KRAS* (codons 12, 13, 61), *BRAF*, and *PIK3CA*		All patients
	Mutant (%)	Wild type (%)	Mutant of any genes (%)	Wild type of all genes (%)	
Total	11 (100)	32 (100)	16 (100)	27 (100)	43 (100)
CR	0 (0)	1 (3.1)	0 (0)	1 (3.7)	1 (2.3)
PR	0 (0)	9 (28.1)	0 (0)	9 (33.3)	9 (20.9)
SD	7 (63.6)	11(34.4)	8 (50.0)	10 (37.0)	18 (41.9)
PD	4 (36.4)	11 (34.4)	8 (50.0)	7 (25.9)	15 (34.9)
RR (%)	0	31.3	0	37.0	23.3
DCR	63.6	65.6	50.0	74.1	65.1
PFS (median)	3.0 M	5.7 M	2.8 M	6.4 M	4.7 M

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *M* months

Cetuximab efficacy

The RR and median PFS (mPFS) according to the presence or absence of gene mutations are shown in Table 4. In the 43 assessable patients, the RR and mPFS correlated with *KRAS*, *BRAF*, and *PIK3CA* mutation status. No responder was observed among the 16 patients with mutations in any one of the three genes, although there were 11 responders among the 27 patients with no gene mutation. In the 27 patients with no detected mutations, objective RR was 40.7 %; in 16 patients with mutated tumors, objective RR was 0 %. In patients with wild-type *KRAS* in codons 12 and 13, *KRAS* in codon 61, *BRAF*, and *PIK3CA* mutations were associated with lack of response.

The mPFS of the wild-type *KRAS* (codon 12 and 13) subgroup was significantly longer than that of mutant *KRAS* (codon 12 and 13) subgroup (5.7 vs. 3.0 months; $P = 0.017$) (Fig. 1a). However, the difference of mPFS between wild-type *KRAS* (codon 12, 13, and 61), *BRAF* and *PIK3CA* subgroup, and mutant subgroup in any of the three genes was considerably more (6.4 vs. 2.8 months; $P = 0.0069$) (Fig. 1b). Consistent results with RR and mPFS were observed in the plot of best response of target lesions and mutation status. Almost all patients with any mutation in *KRAS*, *BRAF*, and *PIK3CA* failed to respond to cetuximab-based treatment (Fig. 2a). No patient in the mutant *KRAS* group had a tumor reduction (Fig. 2b). In contrast, 50 % of the wild-type *KRAS* group had a tumor reduction, including patients with PR and SD (Fig. 2c); 0.06 % of the group with any mutant *KRAS*, *BRAF*, and *PIK3CA* and 56 % of the all wild-type group had a tumor reduction, respectively (Fig. 2d, e). All the four patients with severe progressive disease (more than 40 % tumor increase from baseline) were included in the group with any mutant *KRAS*, *BRAF*, and *PIK3CA* genes. These results indicate the clinical relevance of mutations in these genes in predicting the efficacy of cetuximab-based treatment in patients with mCRC.

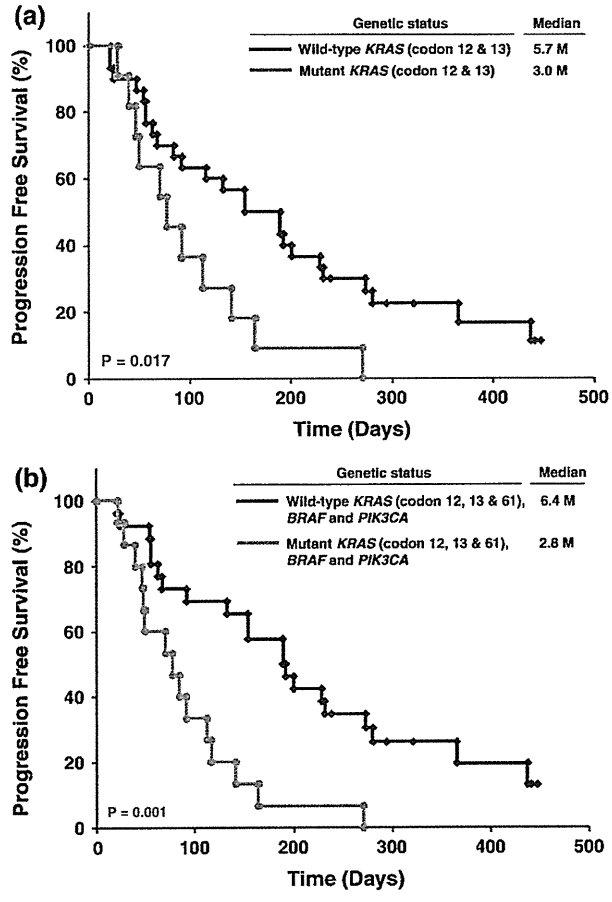

Fig. 1 Kaplan–Meier cumulative progression-free survival (PFS) based on *KRAS*, *BRAF*, and *PIK3CA* mutational status in metastatic colorectal cancer (mCRC) patients treated with cetuximab. **a** Patients with wild-type *KRAS* (codons 12, 13) versus mutant *KRAS*. **b** Patients with all wild-type *KRAS* (codons 12, 13, 61), *BRAF*, and *PIK3CA* versus any mutant *KRAS*, *BRAF*, and *PIK3CA*

Discussion

Our data confirmed that *KRAS* status is a significant predictive marker of cetuximab response in Japanese patients

with mCRC as it is in Caucasians, and the combination of *KRAS*, *BRAF*, and *PIK3CA* analyses improved predictive sensitivity. The wild-type *KRAS* (codons 12 and 13) subgroup showed better clinical outcomes than did the mutant *KRAS* subgroup in terms of RR and mPFS (Fig. 1a). Moreover, the difference of clinical outcome was wider by comparing between the wild-type subgroup in all *KRAS* (codons 12, 13, and 61), *BRAF*, and *PIK3CA* genes and the mutant subgroup in any of the three genes than comparing between the wild-type *KRAS* (codons 12 and 13) and the mutant subgroup (Fig. 1b). Then, combined analysis of the three genes and addition of *KRAS* codon 61 mutation analysis contributed to a better selection of the patients likely to benefit from cetuximab treatment. In contrast, no responders were found among the five patients with tumors harboring either *KRAS* codon 61, *BRAF*, or *PIK3CA* mutations. It is a noteworthy tendency that combination of mutations of the three genes contributes to selecting severely progressive patients who benefit least from anti-EGFR therapy (Fig. 2a). The RR of the wild-type *KRAS* and the RR of the wild-type *KRAS*, *BRAF*, and *PIK3CA* in this study were almost comparable with those of the large-scale analysis in Europeans [15], suggesting that the significance of *KRAS*, *BRAF*, and *PIK3CA* mutations in prediction of cetuximab efficacy is almost identical between Asians and Caucasians. Nevertheless, almost 60 % of patients without any mutations in *KRAS*, *BRAF*, and *PIK3CA* genes still did not respond to cetuximab and suffered tumor progression. These results also suggest that there are other, unidentified molecular response determinants. We analyzed other downstream factors in the EGFR signaling pathway including *NRAS*, *AKT1*, and *PIK3R1*. Although previous reports have shown mutations in *NRAS*, *AKT1*, and *PIK3R1* genes in 2.64 % [15], 6 % [19], and 8.3 % [20] of patients with mCRC, respectively, we did not identify any mutations in these genes. Thus, we could not evaluate the significance of these gene mutations as a biomarker of anti-EGFR therapy because of low prevalence. However, we excluded the possibility that these genes were responsible for the treatment resistance we observed in patients with *KRAS*, *BRAF*, and *PIK3CA* wild-type mCRC. Additional biomarkers are needed to improve the identification of patients who will benefit from cetuximab treatment. One of the candidate biomarkers is the tumor suppressor PTEN protein, which is a negative regulator of PI3-kinase-initiated signaling. The loss of PTEN expression determined by immunohistochemistry has been associated with a lack of response to cetuximab [21, 22].

The *KRAS* mutation frequency in this study was low (27.9 %) in comparison to previous reports (40–50 %). The reason for this lower prevalence is likely the result of clinical bias as a consequence of the retrospective study design. We enrolled patients who received cetuximab as third-line therapy or later just after approval of cetuximab for use in Japan. Initially, the patients were treated with cetuximab without *KRAS* analysis in advance, causing no bias in the population of the *KRAS* mutants. However, after the *KRAS* analysis became available, the patients were treated only if the tumors harbored wild-type *KRAS*. This situation made the mutation frequency of *KRAS* lower than other studies, but also made our data valuable because no further clinical data regarding cetuximab treatment in Japanese patients with *KRAS*-mutant tumors will be available. The *KRAS* mutation frequency in 186 patients with mCRC was also analyzed during this study, including patients who did not receive cetuximab treatment for various reasons. The *KRAS* mutation was found in this population in similar frequency to that described in the previous studies (75/186 = 40.3 %). Moreover, the pattern of *KRAS* mutations was very similar to the previous Caucasian studies [23, 24]. Thus, we concluded that *KRAS* mutation in terms of both frequency and the mutation spectrum does not differ between Japanese and Caucasians. Recently, the *KRAS* G13D mutation has been shown to be associated with better outcome after treatment cetuximab than was observed with other mutations [25]. In this study, three patients with *KRAS* G13D-mutated tumor had no tendency to show better response to cetuximab-based therapy than those with other mutations (Fig. 2c), even though the sample size was low. The prevalence of *BRAF* mutation (4.6 %) was also lower than the reports in Caucasian studies [26], which could be the result of ethnic difference. However, *BRAF* mutations have shown to be a prognostic marker and a predictive marker of anti-EGFR antibody therapy [13]. Then, one of the possible explanations of this lower prevalence is that patients with the *BRAF* mutation become intolerant of additional therapy through multiple lines of chemotherapy, as similarly reported in several studies [15]. The prevalence of *PIK3CA* mutation (4.7 %) was quite lower than that observed in the previous studies (10–20 %). Of the two detected mutations, E542K is one of the three hot-spot mutations (E542K, E545K, and H1047R), whereas E545G is a rare mutation [15, 27]. Large-scale analysis will clarify whether this discrepancy in mutation frequency and spectrum is caused by ethnic differences. The clinical relevance of *PIK3CA* mutations in prediction of the response to anti-EGFR therapy is still controversial. Although most studies do not evaluate the mutation in exons 9 and 20 separately, a recent large European study has shown that only *PIK3CA* mutations in exon 20 but not those in exon 9 are associated with resistance to anti-EGFR antibody. We detected the *PIK3CA* mutation only in exon 9, and the mutated tumor showed no response to cetuximab. Our data indicated the mutations in exon 9 possibly abrogated the effect of cetuximab.

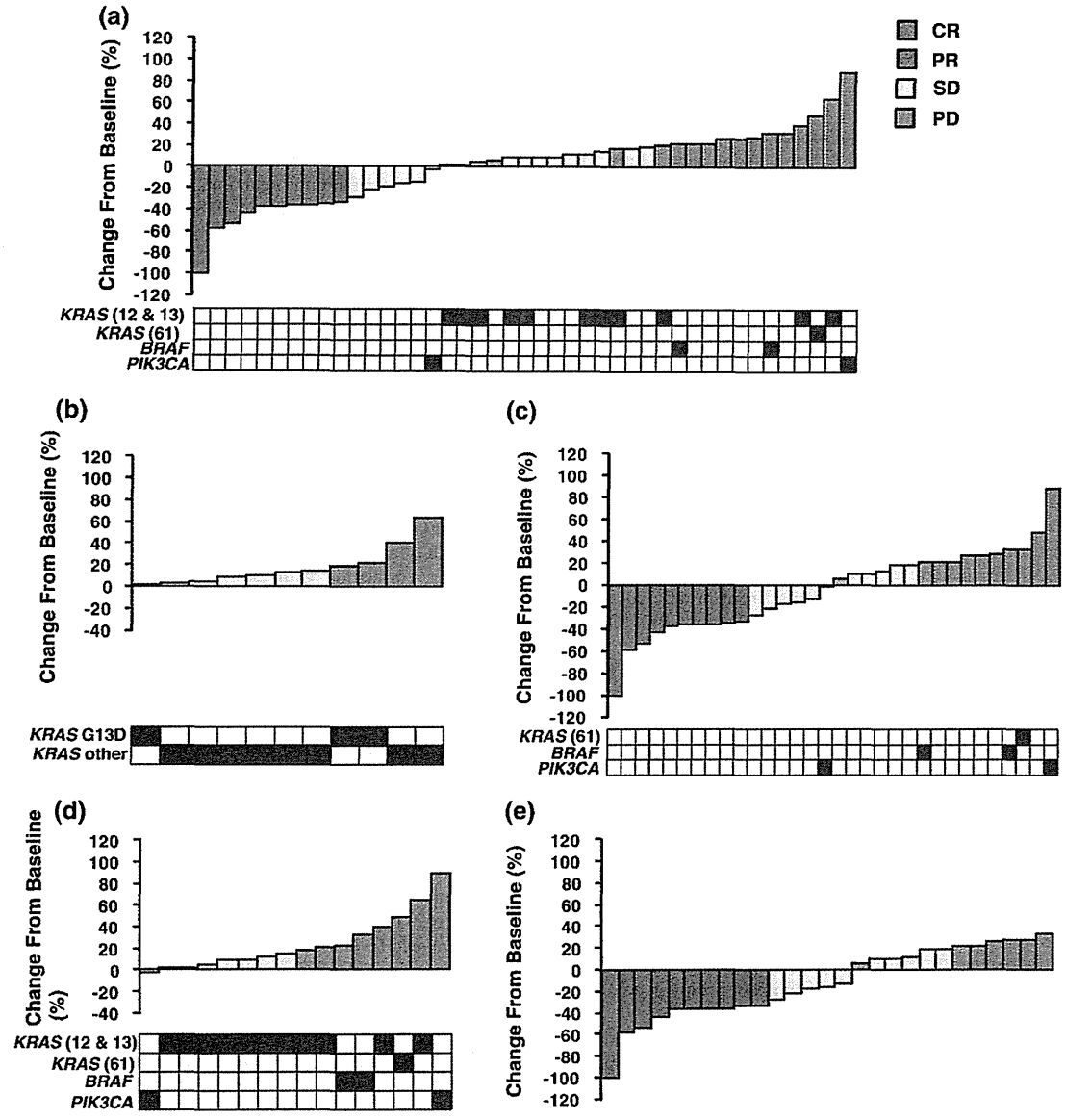

Fig. 2 *Waterfall plots* showing maximal reduction of target lesions based on *KRAS*, *BRAF*, and *PIK3CA* mutational status in mCRC patients treated with cetuximab. **a** All patients. **b** Patients with mutant *KRAS* (codons 12, 13). **c** Patients with wild-type *KRAS* (codons 12, 13). **d** Patients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

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

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

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

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References

1. Hecht JR, Mitchell E, Neubauer MA et al (2010) Lack of correlation between epidermal growth factor receptor status and response to panitumumab monotherapy in metastatic colorectal cancer. Clin Cancer Res 16:2205–2213
2. Di Fiore F, Blanchard F, Charbonnier F et al (2007) Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. Br J Cancer 96:1166–1169
3. De Roock W, Piessevaux H, De Schutter J et al (2008) KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Ann Oncol 19:508–515
4. Lievre A, Bachet JB, Boige V et al (2008) KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol 26:374–379
5. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F et al (2007) Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Res 67:2643–2648
6. Lievre A, Bachet JB, Le Corre D et al (2006) KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 66:3992–3995
7. Amado RG, Wolf M, Peeters M et al (2008) Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 26:1626–1634
8. Karapetis CS, Khambata-Ford S, Jonker DJ et al (2008) K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 359:1757–1765
9. Frattini M, Saletti P, Romagnani E et al (2007) PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. Br J Cancer 97:1139–1145
10. Khambata-Ford S, Garrett CR, Meropol NJ et al (2007) Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol 25:3230–3237
11. Di Nicolantonio F, Martini M, Molinari F et al (2008) Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol 26:5705–5712
12. De Roock W, Lambrechts D, Tejpar S (2009) K-ras mutations and cetuximab in colorectal cancer. N Engl J Med 360:834; author reply 835–836
13. Tol J, Nagtegaal ID, Punt CJ (2009) BRAF mutation in metastatic colorectal cancer. N Engl J Med 361:98–99
14. Prenen H, De Schutter J, Jacobs B et al (2009) PIK3CA mutations are not a major determinant of resistance to the epidermal growth factor receptor inhibitor cetuximab in metastatic colorectal cancer. Clin Cancer Res 15:3184–3188
15. De Roock W, Claes B, Bernasconi D et al (2010) Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol 11:753–762
16. Lynch TJ, Bell DW, Sordella R et al (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 350:2129–2139
17. Han SW, Kim TY, Hwang PG et al (2005) Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. J Clin Oncol 23:2493–2501
18. Paez JG, Janne PA, Lee JC et al (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 304:1497–1500
19. Carpten JD, Faber AL, Horn C et al (2007) A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature (Lond) 448:439–444
20. Jaiswal BS, Janakiraman V, Kljavin NM et al (2009) Somatic mutations in p85alpha promote tumorigenesis through class IA PI3K activation. Cancer Cell 16:463–474
21. Loupakis F, Pollina L, Stasi I et al (2009) PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer. J Clin Oncol 27:2622–2629
22. Negri FV, Bozzetti C, Lagrasta CA et al (2010) PTEN status in advanced colorectal cancer treated with cetuximab. Br J Cancer 102:162–164
23. Andreyev HJ, Norman AR, Cunningham D et al (1998) Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. J Natl Cancer Inst 90:675–684
24. Andreyev HJ, Norman AR, Cunningham D et al (2001) Kirsten ras mutations in patients with colorectal cancer: the "RASCAL II" study. Br J Cancer 85:692–696
25. De Roock W, Jonker DJ, Di Nicolantonio F et al (2010) Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. JAMA 304:1812–1820
26. Barault L, Veyrie N, Jooste V et al (2008) Mutations in the RAS–MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. Int J Cancer 122:2255–2259
27. Ogino S, Nosho K, Kirkner GJ et al (2009) PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer. J Clin Oncol 27:1477–1484
28. Cunningham D, Humblet Y, Siena S et al (2004) Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 351:337–345

「ここまで進歩した外来がん化学療法」

消化器癌（大腸癌・胃癌）

東北大学加齢医学研究所
臨床腫瘍学分野准教授
加藤俊介

大腸癌

治療成績が向上した大腸癌

消化器癌に対する化学療法の中で近年最も治療成績が向上したものは大腸癌であろう．大腸癌ではフッ化ピリミジン系薬剤，オキサリプラチン，イリノテカンの3種類の殺細胞効果薬剤に加えて，血管新生阻害薬であるベバシズマブ，腫瘍細胞のKRAS遺伝子に変異がなければ抗EGFR抗体薬であるセツキシマブ，パニツムマブなどの分子標的治療薬が使用される[1]．

患者状態が良好である場合は，一般的にこれら薬剤を組み合わせた併用療法が行われ，FOLFOX療法（5-FU＋l-ロイコボリン＋オキサリプラチン）やFOLFIRI療法（5-FU＋l-ロイコボリン＋イリノテカン）に分子標的治療薬を上乗せして一次治療を行い，効果が見られなくなった場合や有害事象が問題となった場合，殺細胞効果薬剤を変更して二次治療を行うケースが通常である．また最近，ベバシズマブについては一次治療で病勢進行が見られた場合，二次治療でもベバシズマブを継続して使用することにより生存期間の延長が得られたことが報告されている．

経口薬レジメンの登場

大腸癌の外来化学療法を行う上で中心静脈アクセスポート（CVポート）は重要な働きを担ってきた．これはFOLFOX療法やFOLFIRI療法など殺細胞効果薬剤による化学療法の骨格に，5-FUの持続点滴（46時間）が含まれているためである．そのため，外来化学療法を行っている施設では，CVポートの適切な管理についての患者指導が必要であった．

近年5-FU持続点滴の代わりに経口フッ化ピリミジン系薬剤の一種であるカペシタビンやS-1による，CVポートを使用しないレジメンも開発されてきている．特にカペシタビンでは，オキサリプラチンとの併用療法であるCapeOX（XELOX）療法が日常診療でも行われるようになっている（図1）[2]．経口薬は服薬

コンプライアンスに注意が必要だが，CVポートを造設せずに済む利点がある．

各薬剤の有害事象と注意点

カペシタビンには手掌や足裏が赤く腫れ上がる手足症候群と呼ばれる有害事象があり，患者の生活の質を落とすことがある．普段からの保湿クリーム塗布などのスキンケア指導は重要であるが，状況によってはカペシタビンの休薬・減量やステロイド外用が必要になる．

オキサリプラチンの特徴的な有害事象はしびれを主体とした末梢神経障害である．寒冷刺激で増強する瞬間的な神経毒性に加え，用量依存的に蓄積性に末梢神経障害が増悪するため，日常生活に支障を来す前に休薬が必要になることに注意する．

分子標的治療薬では，今までの殺細胞効果薬剤にはない特徴的な有害事象が見られる．血管新生阻害薬であるベバシズマブは高血圧，蛋白尿，出血などが見られるほか，頻度は低いものの（1%以下）心筋梗塞や脳梗塞などの動脈血栓症，消化管穿孔など致死的な有害事象もあるため，これらの症状を疑わせる所見があれば，高次医療機関での早急な対応が必要になることに留意するべきである．

抗EGFR抗体は致死的な有害事象は少ないものの，ほぼ全例において初回治療後から早期にニキビ様の皮疹が出現する．治療を重ねることで皮疹は広がり，さらには皮膚乾燥や爪囲炎など患者の生活の質の低下を来すことがあるので，普段からの保湿剤によるスキンケア指導は重要である．

最近の重要なstudyとその意義

ベバシズマブ，カペシタビンの有用性

・NO16966試験は未治療の切除不能・再発進行大腸癌を対象としてフッ化ピリミジン系薬剤とオキサリプラチンの化学療法〔FOLFOX4療法あるいはCapeOX（XELOX）療法〕に対するベバシズマブの上乗せを検証した第Ⅲ相試験．

主要評価項目は無増悪生存期間．

・化学療法単独群の無増悪生存期間の中央値8.0カ月に対し，ベバシズマブと化学療法の併用群では9.4カ月と有意に無増悪生存期間の延長は認められたが，全生存期間の延長は認められなかった．

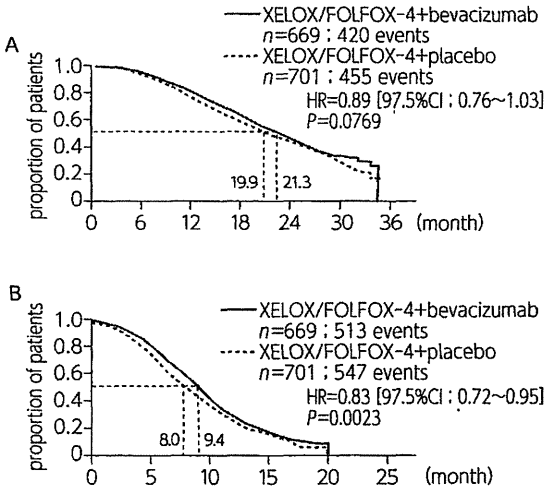


図1　NO16966試験の全生存期間（A）と無増悪生存期間（B）　（文献[2]より引用）

胃癌

一次治療ではS-1とシスプラチン併用が標準治療

- 胃癌に対する有用な殺細胞性抗癌剤は，その作用機序からフッ化ピリミジン系薬剤，シスプラチン，タキサン系薬剤，イリノテカンの4種類の薬剤が使用される．さらに胃癌全体の10～20%を占めるHER2過剰発現胃癌には分子標的薬剤トラスツズマブも用いられる．
- これまで経口フッ化ピリミジン系薬剤であるS-1とシスプラチンの併用療法は，S-1単剤と比較し有意に生存期間を延長させることが報告され，一次治療の標準治療（図2）[3]とみなされてきたが，近年S-1とドセタキセルの併用療法もS-1単剤と比較し有意に生存期間を延長させることが報告された[4]．
- また，二次治療においてイリノテカン，タキサン系薬剤の使用による生存期間の延長が報告されている[5]．

注意したい有害事象

(1) 経口フッ化ピリミジン系薬剤S-1

- S-1は5-FUの誘導体テガフール，5-FUの分解を阻害するギメラシル，5-FUによる消化管障害を軽減させるオテラシルの3成分が配合された薬剤である．
- S-1の主な有害事象は骨髄抑制や口内炎や下痢などの消化管毒性のほか，近年は涙管閉塞による流涙などが問題となっている．有害事象は比較的軽度であり，経口薬のため外来でも使いやすいが，腎機能低下症例においてはギメラシルの排泄が遅れることにより5-FUの代謝が遅れ，有害事象が強くなる．

(2) シスプラチン投与が入院から外来へ

- S-1との併用で使用されることが多いシスプラチンはプラチナ製剤であり，腎排泄である．悪心・嘔吐を強く誘発し，かつ薬剤の排泄のための補液が必要であることから，シスプラチンの投与前後は入院で治療を行う施設が多い．
- 近年有力な制吐薬であるアプレピタントが本邦でも使用可能となったことや『制吐薬適正使用ガイドライン』の普及で悪心・嘔吐に対するマネジメントが浸透してきたこと，さらに補液へのマグネシウム付加により腎毒性の軽減が可能であることが報告され，外来で化学療法を行う施設も増えている．
  シスプラチンより悪心・嘔吐，腎毒性の点でマネジメントがしやすいオキサリプラチン＋S-1併用療法の有効性について，シスプラチン＋S-1併用療法と比較する第Ⅲ相試験（非劣性試験）が行われており，その結果次第ではシスプラチンの代用となることも期待されている．

(3) イリノテカン，タキサン系薬剤などの有害事象

- イリノテカン，タキサン系薬剤は点滴時間が短いことから，外来化学療法でも

使用されやすい薬剤である.

イリノテカンの催吐性は中等度リスクであり，その他下痢，骨髄抑制が有害事象として挙げられる．イリノテカンは*UGT1A1*の遺伝子多型を調べることにより，骨髄抑制の有害事象をある程度予測でき，外来での治療をより安全に行うことができる．下痢は投与直後と約1週間後に起きるものが特徴的であり，胆汁排泄薬剤であることから投与期間中の規則正しい排便習慣が重要である.

タキサン系薬剤は催吐リスクが低く，外来でも使用しやすいが，パクリタキセルは過敏症を引き起こすことがあるので注意が必要である．またパクリタキセルを分割して投与する場合は骨髄抑制が軽度であるが，ドセタキセル単剤投与の場合は一括投与のため，比較的早期（約1週間後）に起こる骨髄抑制に注意する.

シスプラチン，イリノテカンなどの催吐リスクが中等度以上の薬剤については遅延性の悪心・嘔吐防止のためステロイドを使用することが原則となっている．パクリタキセルも過敏症防止のためステロイドを使用することから，糖尿病を合併している患者については血糖コントロールが悪くなる可能性があり，やはり注意が必要である.

最近の重要なstudyとその意義

S-1とシスプラチン併用療法の有用性

- SPIRITS試験は未治療の切除不能・再発進行胃癌を対象としてS-1単剤療法とS-1＋シスプラチン併用療法の有効性について直接比較した第Ⅲ相試験.
- 主要評価項目は全生存期間.
- 全生存期間の中央値はS-1単剤療法群11.0カ月だったのに対し，S-1＋シスプラチン併用療法群では13.0カ月，無増悪生存期間の中央値はそれぞれ4.0カ月と6.0カ月であり，いずれも有意にS-1＋シスプラチン併用療法群で良好な成績であった.

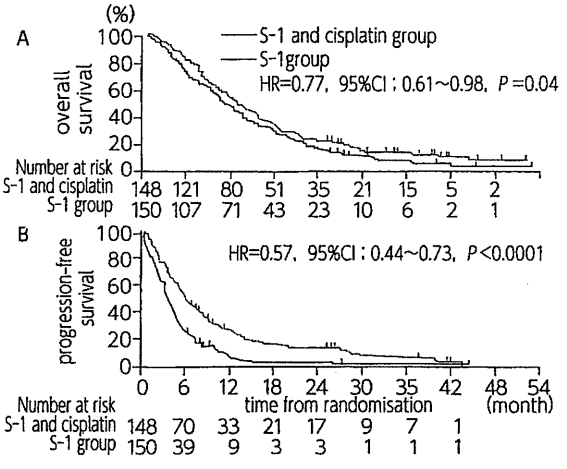


図2 SPIRITS試験の全生存期間（A）と無増悪生存期間（B）
（文献[3]より引用）

文 献

1) Eng C : Oncologist 15 : 73, 2010.
2) Saltz LB, et al : J Clin Oncol 26 : 2013, 2008.
3) Koizumi W, et al : Lancet Oncol 9 : 215, 2008.
4) Yoshida K, et al : ESMO2012, abs#LBA19.
5) Kang JH, et al : J Clin Oncol 30 : 1513, 2012.

● 特 集 ● 新しい分子標的治療薬による治療戦略

大腸がんに対する新しい分子標的薬（レゴラフェニブとアフリバセプト）

加藤 俊介*

〔*Jpn J Cancer Chemother* 40(1): 6-9, *January*, 2013〕

New Molecular-Targeted Agents for Colorectal Cancer—Regorafenib and Aflibercept: Shunsuke Kato (*Dept. of Clinical Oncology, Institute of Development, Aging and Cancer, Tohoku University*)

Summary

Since the approval of bevacizumab, cetuximab, and panitumumab, development of novel molecular-targeted agents for colorectal cancer has been awaited. Recent phase Ⅲ trials revealed that new molecular-target agents, regorafenib and aflibercept, are useful for previously treated mCRC. Regorafenib is an oral multi-kinase inhibitor which targets angiogenic, stromal and oncogenic receptor tyrosine kinase (RTK). Aflibercept is a recombinant fusion protein that consists of vascular endothelial growth factor (VEGF)-binding portions from the extracellular domains of human VEGF receptors 1 and 2, fused to the Fc portion of the human IgG1 immunoglobulin. They are different in structure, but an anti-angiogenic effect is one of the main mechanisms of action they have in common. Consistent with the 18147 ML study, bevacizumab beyond progression (BBP), anti-angiogenic therapies are important in any line of chemotherapy for colorectal cancer. **Key words**: Colorectal cancer, Regorafenib, Aflibercept, Angiogenesis, **Corresponding author**: Shunsuke Kato, Department of Clinical Oncology, Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan

要旨 大腸がんに対する分子標的治療薬は，抗VEGF抗体ベバシズマブと抗EGFR抗体セツキシマブ，パニツムマブの開発以来しばらくとどまっていたが，近年の第Ⅲ相試験の結果により，レゴラフェニブとアフリバセプトという新しい分子標的薬剤の有用性が証明された。レゴラフェニブはマルチターゲット型受容体チロシンキナーゼ阻害剤であり，アフリバセプトはVEGF-A，VEGF-BおよびPIGF（胎盤成長因子）と結合・阻害を起こす融合抗体蛋白質である。これら薬剤は小分子化合物と可用性結合蛋白製剤という構造上の違いはあるが，主な作用機序の一つに血管新生阻害効果がある点で共通する。これら薬剤において治療効果がみられたことは，bevacizumab beyond progression (BBP) のコンセプトがML18147試験で証明されたことと合わせ，大腸がん治療における血管新生阻害の意義が一次治療からそれ以降の治療期間を通じて常に重要であることが示されたものとも考えられる。

はじめに

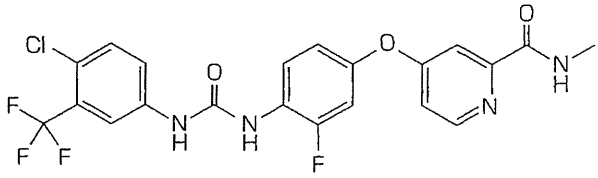
血管新生は固形腫瘍の増殖に重要な働きを示す。そのなかでも腫瘍増殖の制御にはVEGFの継続的な抑制が重要であることは，これまでマウスモデルなどの前臨床データより報告されてきた[1,2]。しかし実臨床においては，ベバシズマブを含む一次治療に耐性化した後にさらに継続してベバシズマブを使用するいわゆるbevacizumab beyond progression (BBP) については，大規模な観察試験であるBRiTE試験による後ろ向き研究しか報告がなく，その是非については長年議論を呼んでいた。

しかし2012年ASCO総会において，ベバシズマブを含む標準的な一次治療に不応となった切除不能な大腸がん患者を対象としたML18147試験が，BRiTE試験ほどのインパクトはなかったもののベバシズマブの継続利用の有用性を証明した[3]。さらに，2012年に相次いで発表された大腸がんの二次，三次治療以降患者を対象とした二つの新規薬剤の大規模な第Ⅲ相試験（CORRECT試験のレゴラフェニブ，VELOUR試験のアフリバセプト）の結果も，血管新生阻害がどのラインにおいても重要であることを示したものと考えられる。

* 東北大学加齢医学研究所・臨床腫瘍学分野

連絡先: 〒980-8575 宮城県仙台市青葉区星陵町4-1 東北大学加齢医学研究所・臨床腫瘍学分野
加藤 俊介

レゴラフェニブ



ソラフェニブ

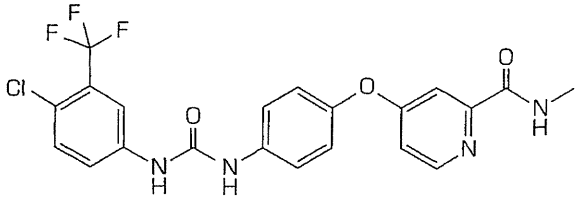


図 1　レゴラフェニブとソラフェニブの構造の違い

表 1　レゴラフェニブとソラフェニブの標的分子に対する阻害活性

標的分子	レゴラフェニブ IC_{50}(nM)±SD	ソラフェニブ IC_{50}(nM)±SD
c-RAF	2.5±0.6	6±3
BRAF	28±10	22±6
$BRAF^{V600E}$	19±6	38±9
VEGFR-1	13±0.4	NA‡
VEGFR-2	4.2±1.6†	90±15
VEGFR-3	46±10†	20±6
TIE-2	311±46	NA‡
PDGFR-β	22±3	57±20
FGFR1	202±18	580±100
KIT	7±2	68±21
RET	1.5±0.7	NA‡
Flt-3	NA‡	58±20

†: マウス VEGFR に対する阻害活性を示す
‡: not available

Ⅰ. レゴラフェニブ

レゴラフェニブは経口マルチキナーゼ阻害剤として開発された薬剤である。その構造は先行薬であるソラフェニブに類似しており（図1），標的とする分子もお互いに類似しているが，レゴラフェニブは VEGFR や TIE-2 など血管新生にかかわる分子群や変異型 KIT，RET に対してより特異的にキナーゼ活性を阻害するとされており（表1）[4]，実際に 2012 年 ASCO において前治療歴のある GIST に対してもその有効性が証明されている。

転移性大腸がんについては，38 例を対象に行われた第Ⅰ相試験の拡大コホートでは，評価可能病変を有する 27 名の患者に対しレゴラフェニブ 160 mg/日を 3 週投与 1 週休薬のスケジュールで投与した結果，1 名の PR を含む 20 例（74%）で 7 週間以上の病勢コントロールが可能であったと報告され[5]，引き続き第Ⅲ相国際共同治験 CORRECT 試験が計画された[6]。

この第Ⅲ相試験は，標準的治療，すなわちフッ化ピリミジン，イリノテカン，オキサリプラチンの殺細胞効果薬剤 3 剤とベバシズマブ，さらに *KRAS* 野生型症例に対しては，抗 EGFR 抗体を含むすべての薬剤に対して不応になった切除不能再発大腸がん患者 760 名（日本人 100 名を含む）を対象とし，レゴラフェニブ投与群とプラセボ投与群を 2：1 に無作為化割り付けし，主要評価項目は全生存期間に設定された。その結果，全生存におけるハザード比は 0.77（95%信頼区間: 0.64-0.94，p=0.0052），生存期間の中央値はレゴラフェニブ群 6.4 か月，プラセボ群が 5.0 か月と，レゴラフェニブ投与により生存期間は有意に延長した（表2）。また，奏効率は 1.0%しかみられないものの，副次的評価項目である無増悪生存期間，病勢コントロール率においても両群間に有意な差を認めた。また，*KRAS* 変異の有無による治療効果の違いは認められなかったと報告されている。

一方，レゴラフェニブによる主な有害事象は，手足皮膚反応，倦怠感，高血圧，下痢など他のマルチキナーゼ阻害剤同様の有害事象が観察されている。大腸がんに対してこれまで多くのマルチキナーゼ阻害剤が殺細胞効果薬剤との上乗せを証明することができなかったが，その理由として有害事象のためベースとなっている殺細胞効果薬剤を減量せざるを得なかったことがあげられている。本試験のレゴラフェニブは単剤投与であったが，有害事象による治験薬中止はレゴラフェニブ群で 8.2%に上っており，標準治療を十分行ってきた患者に対しては体調管理に十分注意しながら投与することが肝要であると思われる。

Ⅱ. アフリバセプト

ベバシズマブは VEGF-A の中和抗体であるのに対して，アフリバセプトは VEGF-A，VEGF-B と結合する VEGFR-1 の細胞外ドメイン中の二番目のイムノグロブリン（Ig）ドメインと，VEGFR-2 の三番目の Ig ドメインを融合させた抗体蛋白質（VEGF trap）である（図2）[2]。アフリバセプトは VEGF-A と VEGF-B および PIGF（胎盤成長因子）に結合し，それぞれの受容体への結合を阻害することにより血管新生阻害に働く。多くのヒト腫瘍においては VEGF-A の高発現が認められているが，いくつかの癌種においては VEGF-B の発現レベルの上昇が予後不良因子として関連することや，抗 VEGF 治療により VEGF-A シグナルを増強する PIGF の発現レベルが上がってくることがこれまで報告されており，アフリバセプトはベバシズマブ耐性症例に対しても治療効果が期待されていた。

そのなかで行われたVELOUR試験は，オキサリプラチンによる治療歴のある転移を有する大腸がん患者を対象とし，FOLFIRI療法へのアフリバセプトの上乗せ効果を検証する第Ⅲ相試験として行われた（表3）[7]。主要評価項目である全生存期間，副次評価項目である無増悪生存期間，奏効率でも，有意な差をもってアフリバセプトの有効性が証明された。有害事象については，血管新生阻害薬に特徴的な高血圧，出血，蛋白尿が多く観察された他，FOLFIRI療法にみられるような消化器症状や血液毒性も増強する傾向がみられた。

また，この試験は前治療でベバシズマブの使用歴のある患者も含まれていたが，層別解析結果ではベバシズマブの使用歴のある群においても，アフリバセプトを併用したほうが全生存期間，無増悪生存期間を改善する傾向が示された。

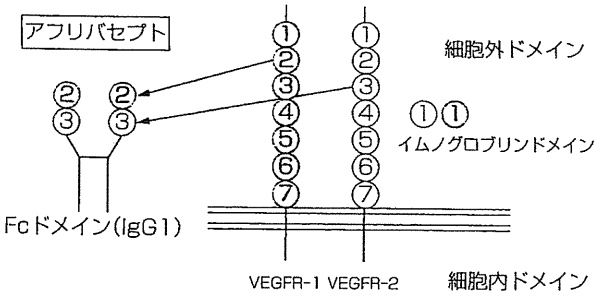


図2 アフリバセプトの構造（文献[2]参照）
VEGFR-1, -2ともに七つの構造の異なるイムノグロブリンドメインをもっている。

表2 CORRECT試験の結果（文献[6]参照）

	レゴラフェニブ	プラセボ	ハザード比
患者数	505	255	
全生存期間中央値（月）	6.4	5.0	0.77
(95%信頼区間)	(5.9-7.3)	(4.4-5.8)	(0.64-0.94)
無増悪生存期間中央値（月）	1.9	1.7	0.49
(95%信頼区間)	(1.9-2.1)	(1.7-1.7)	(0.42-0.58)
奏効率（%）	1.0	0	
病勢コントロール率（%）	44.8	15.3	
主な有害事象（%）（>Grade 3）			
手足症候群	46.6 (16.6)	7.5 (0.4)	
疲労	47.4 (9.6)	28.1(5.1)	
高血圧	27.8 (7.2)	5.9 (6.7)	
皮疹	26.0 (5.8)	4.0 (0)	
下痢	33.8 (7.2)	8.3 (0.8)	
口内炎	27.2 (3.0)	3.6 (0)	
血小板減少	12.6 (2.8)	2.0 (0.4)	
毒性による中止（%）	8.2	1.2	

表3 VELOUR試験の結果（文献[7]参照）

	アフリバセプト/FOLFIRI	プラセボ/FOLFIRI	ハザード比
患者数	612	614	
全生存期間中央値（月）	13.5	12.06	0.817
(95%信頼区間)	(12.52-14.95)	(11.07-13.11)	(0.713-0.937)
無増悪生存期間中央値（月）	6.9	4.67	0.758
(95%信頼区間)	(6.51-7.20)	(4.21-5.36)	(0.661-0.869)
奏効率（%）	19.8	11.2	
(95%信頼区間)	(16.4-23.2)	(8.5-13.8)	
主な有害事象（%）（>Grade 3）			
高血圧	41.4 (19.3)	10.7 (1.5)	
出血	37.8 (3.0)	19.0 (1.7)	
蛋白尿	62.2 (7.8)	40.7 (1.2)	
動脈血栓	2.6 (1.8)	1.5 (0.5)	
消化管穿孔	0.5 (0.5)	0.5 (0.4)	
下痢	69.2 (19.3)	56.5 (7.8)	
口内炎，潰瘍	54.8 (13.8)	34.9 (5.0)	
気分変調	25.4 (0.5)	3.3 (0)	
毒性による中止（%）	26.8	12.1	

表 4 血管新生にかかわる分子（文献[2]参照）

経路	血管新生因子	受容体
VEGF	VEGF-A[†,§]	VEGFR-1[‡], VEGFR-2[‡], NRP-1, NRP-2
	VEGF-B[§]	VEGFR-1[‡], NRP-1, NRP-2
	PIGF[§]	VEGFR-1[‡], NRP-1, NRP-2
	VEGF-C	VEGFR-2[‡], VEGFR-3[‡], NRP-1, NRP-2
	VEGF-D	VEGFR-2[‡], VEGFR-3[‡], NRP-1, NRP-2
Ang	Ang-1	TIE-2[‡]
	Ang-2	TIE-2[‡]
	Ang-4	TIE-2[‡]

[†]：ベバシズマブ，[‡]：レゴラフェニブ，[§]：アフリバセプトの主な標的を示す

今後の課題

これまでの試験結果から，大腸がんにおいては血管新生阻害治療がどのラインにおいても有効であることがほぼ確実になりつつある。しかし，ここで問題となるのが，たとえばセカンドラインにアフリバセプトを使用するか，ML18147試験結果に基づきベバシズマブを継続使用するかである。二次治療にベバシズマブを使用するかアフリバセプトを使用するかについては，直接の比較が行われていないため治療の優劣を決めることは難しい。さらには，*KRAS*野生型に対しては抗EGFR抗体をどの時点で使用するかもかかわってくる。

今後，血管新生阻害効果を有する薬剤については，表4にあげたようなそれぞれの薬剤の標的分子のプロファイルの違いを考慮したバイオマーカー探索と，症例に応じた個別化医療の開発が期待される。

文 献

1) Bagri A, Berry L, Gunter B, *et al*: Effects of anti-VEGF treatment duration on tumor growth, tumor regrowth, and treatment efficacy. *Clin Cancer Res* **16**(15): 3887-3900, 2010.
2) Gaya A and Tse V: A preclinical and clinical review of aflibercept for the management of cancer. *Cancer Treat Rev* **38**(5): 484-493, 2012.
3) Arnold D, Andre T, Bennouna J, *et al*: Bevacizumab (BEV) plus chemotherapy (CT) continued beyond first progression in patients with metastatic colorectal cancer (mCRC) previously treated with BEV plus CT: results of a randomized phase Ⅲ intergroup study (TML study). *ASCO Annual Meeting. J Clin Oncol* **30**(*Suppl*): abstr CRA3503, 2012.
4) Strumberg D and Schultheis B: Regorafenib for cancer. *Expert Opin Investig Drugs* **21**(6): 879-889, 2012.
5) Strumberg D, Scheulen ME, Schultheis B, *et al*: Regorafenib (BAY 73-4506) in advanced colorectal cancer: a phase I study. *Br J Cancer* **106**(11): 1722-1727, 2012.
6) Grothey A, Sobrero AF, Siena S, *et al*: Results of a phase Ⅲ randomized, double-blind, placebo-controlled, multicenter trial (CORRECT) of regorafenib plus best supportive care (BSC) versus placebo plus BSC in patients (pts) with metastatic colorectal cancer (mCRC) who have progressed after standard therapies. *2012 Gastrointenstinal Cancer Symposium. J Clin Oncol* **30**(*Suppl* 4): abstr LBA385, 2012.
7) Van Cutsem E, Tabernero J, Lakomy R, *et al*: Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase Ⅲ randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. *J Clin Oncol* **30**(28): 3499-3506, 2012.

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

Shunsuke Kato[a] Hideaki Andoh[e] Makio Gamoh[f] Takuhiro Yamaguchi[b] Yasuko Murakawa[g] Hideki Shimodaira[a] Shin Takahashi[a] Takahiro Mori[c] Hisatsugu Ohori[a] Shun-ichi Maeda[h] Takao Suzuki[d] Satoshi Kato[i] Shoko Akiyama[a] Yuka Sasaki[b] Takashi Yoshioka[j] Chikashi Ishioka[a] on behalf of Tohoku Clinical Oncology Research and Education

[a]Institute of Development, Department of Clinical Oncology, Aging and Cancer, Tohoku University, [b]Department of Biostatistics, Tohoku University School of Medicine, [c]Tohoku University Hospital Cancer Center, and [d]Sendai Medical Center, Sendai, [e]Nakadori General Hospital, Akita, [f]South Miyagi Medical Center, Shibata-gun, [g]Miyagi Cancer Center, Medeshima, [h]Hachinohe City Hospital, Hachinohe, [i]Iwate Prefecture Central Hospital, Morioka, and [j]Department of Clinical Oncology, Faculty of Medicine, Yamagata University, Yamagata, Japan

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

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

Introduction

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

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Chikashi Ishioka
4-1 Seiryo-machi
Aoba-ku, Sendai 980–8575 (Japan)
E-Mail chikashi@idac.tohoku.ac.jp

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

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

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

Patients and Methods

Patient Eligibility

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

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

Treatment Methods

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

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

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

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

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

Interim Analysis about Safety

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

Statistical Analysis

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

Results

Patient Background

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

Safety Verification Test (Step 1)

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

Safety Verification Trial (Step 2)

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

Comparison of Efficacy

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

Table 1. Characteristics of patients

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

ECOG = Eastern Cooperative Oncology Group.

Discussion

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