Table 1. Clinicopathologic features of CRC patients by CIMP marker status of the cancerous tissue

Characteristic	Total (n = 94)	CIMP positive, n = 26 (27.7)	CIMP negative, n = 68 (72.3)	*p*
Age (yr)				
Mean	65.2	65.8	64.9	0.66
Range	35–86	45–79	35–86	
Gender				
Female	49 (52.1)	14 (28.6)	35 (71.4)	1.00
Male	45 (47.9)	12 (26.7)	33 (73.3)	
Location[1]				
Proximal	40 (42.6)	17 (42.5)	23 (57.5)	<0.01
Distal	54 (57.4)	9 (16.7)	45 (83.3)	
Stage				
I or II	40 (42.6)	9 (22.5)	31 (77.5)	0.36
III or IV	54 (57.4)	17 (31.5)	37 (68.5)	
Tumor differentiation				
Well/Moderate	86 (91.5)	21 (24.4)	65 (75.6)	0.03
Por	8 (8.5)	5 (62.5)	3 (37.5)	
KRAS mutation				
+	32 (34.0)	9 (28.1)	23 (71.9)	1.00
−	62 (66.0)	17 (27.4)	45 (72.6)	
BRAF mutation				
+	6 (6.4)	5 (83.3)	1 (16.7)	<0.01
−	88 (93.6)	21 (23.9)	67 (76.1)	

Values in parentheses indicate percentages.
[1]Proximal—cecum, ascending and transverse colon; Distal—descending and sigmoid colon and rectum.

constructed using Minitab and TreeView software, respectively. To avoid an artificial effect of excess signal values, signal ratios of greater than 10 were defined as 10 for the clustering analysis.

KRAS, *BRAF* and *p53* genes mutation

Mutations in the *KRAS* gene (codons 12 and 13) and *BRAF* gene (codon 600) were determined by the pyrosequencing method as previously reported.[20,21] Mutations in the *p53* gene (exons 5–8) were determined by direct sequencing analysis.[16] The PCR primer sequences and sequencing primer sequences used are listed in Supporting Information Table 1.

Statistical analysis

All statistical analyses were conducted using StatView for Windows (version 5.0). Associations between methylation status and clinicopathological features were analyzed by an unpaired *t* test (Student *t* test or Welch *t* test) and Fisher's exact test. All reported *p* values were two-sided, with $p < 0.05$ being considered statistically significant. Pearson and Spearman tests were used to determine correlations, with significance set at $p < 0.05$; *r* represents the measure of the relationship between two variables and varies from −1 to +1. Disease-free survival curves were generated with the Kaplan-Meier method. The log-rank test was used to estimate disease-free survival. Disease-free survival was calculated starting from the date of surgical procedure to the date of finding new metastatic lesion or local recurrences from primary CRC.

Results

Relationship between CIMP status and clinicopathological features in CRCs

The DNA methylation status of 94 CRCs was examined by pyrosequencing analysis. Methylation status was analyzed as both continuous variable (methylation level) and categorical variable. Genes with methylation levels greater than 15% were considered methylation-positive, since lower values could not be easily distinguished from background.[6,13,22] Samples with simultaneous methylation of at least two of the five classical CIMP markers (*hMLH1*, *MINT1*, *MINT2*, *MINT31* and *p16*) were considered CIMP-positive.[3] Using this criterion, 26 (27.7%) CRCs were classified as CIMP-positive (Table 1). On comparing the clinicopathological features of the CIMP-positive and CIMP-negative groups, we found that the CIMP-positive group was significantly associated

with proximal location, poor differentiation and *BRAF* mutation ($p < 0.01$, $p = 0.03$ and $p < 0.01$, respectively).

Quantitative methylation analysis in the proximal and distal colon

The majority of CIMP-negative CRCs appears to be located in the distal colon. To assess whether DNA methylation of genes other than the CIMP markers is affected by location, we first quantitatively examined methylation levels in the 94 corresponding normal-appearing colonic mucosae and compared the levels in the proximal and distal colon (Fig. 1*a*). To avoid sampling bias, normal-appearing mucosae of CRC patients were sampled from two distinct regions, 2 cm and 10 cm from the cancer. Because methylation levels of the two regions were highly consistent (Supporting Information Table 3), we will hereafter use the average methylation data. Methylation levels of *RASSF1A* and *SFRP1* were significantly higher in the distal than the proximal normal-appearing mucosae ($p < 0.01$ and $p < 0.05$, respectively). Methylation levels of *RUNX3* and *SFRP5* were 15% at most and showed no difference regardless of location. The classical CIMP markers exhibited very low levels of methylation. These methylation patterns were identical in the normal-appearing mucosae from the 38 colon polyp patients (Fig. 1*a*), suggesting that the accumulation of DNA methylation in certain genes is an early event known to be a field defect that occurs during tumorigenesis.[13]

In cancerous tissues, substantially increased methylation was detected in all of the genes examined (Fig. 1*b*). Methylation levels of *RASSF1A* were significantly higher in the distal than in the proximal CRCs, as was also observed in the normal-appearing mucosae ($p < 0.05$, Fig. 1*b*). On the other hand, methylation levels of the four classical CIMP markers, *MLH1*, *MINT1*, *MINT2* *MINT31*, and a newly proposed CIMP marker, *RUNX3*[23], were significantly higher in the proximal than the distal CRCs. We also examined three genes, *MGMT*, *RASSF1A* and *SFRP1*, in 22 colon polyps (Fig. 1*b*). Several distal colon polyps showed higher methylation levels of *RASSF1A* and *MGMT* than proximal colon polyps, though the difference was not statistically significant.

These observations were also reproducible when CIMP-negative distal CRCs and CIMP-positive proximal CRCs were compared (Supporting Information Fig. 1). *RASSF1A* was most frequently methylated in distal CIMP-negative CRCs (51%, Fig. 1*c*). We also found that the methylation levels of *RASSF1A* were significantly higher in CIMP-negative than in CIMP-positive CRCs (mean, 18.3%; 95% CI, 14.6–22.0 *vs.* mean, 15.8%; 95% CI, 7.0–24.6; $p = 0.03$). In contrast, the five classical CIMP markers, as well as a new CIMP marker, *RUNX3*, were remarkably methylated in CIMP-positive CRCs. Five of six (83.3%) CRC cases with *BRAF* mutations fell into the proximal CIMP-positive group. Similarly, *RASSF1A* and *MGMT* were more frequently methylated in distal than in proximal colon polyps (Fig. 1*d*).

Concomitant methylation of *MGMT*, *RASSF1A* and *SFRP1* is correlated with age in distal normal-appearing mucosa from CRC cases

Substantial methylation of *MGMT*, *RASSF1A* and *SFRP1* was detected in normal-appearing mucosa from both CRC and colon polyp cases. We determined whether methylation of those three genes is associated with age and might occur concomitantly with methylation of the other loci. In distal normal-appearing mucosa from CRC cases, methylation levels of the three genes were significantly correlated with patient age (Pearson's correlation coefficients, *RASSF1A*, $r = 0.53$, $p < 0.01$; *SFRP1*, $r = 0.36$, $p < 0.01$; and *MGMT*, $r = 0.37$, $p < 0.01$), as well as with one another (all correlations with $p < 0.01$, Fig. 2*a*). In contrast, methylation levels of the genes in the proximal normal-appearing mucosa did not show strong correlations with patient age or with one another as was found in the distal colon (Fig. 2*a*).

In the normal-appearing mucosa from distal colon polyp cases, methylation levels of *RASSF1A* and *SFRP1* were significantly correlated with patient age (*RASSF1A*, $r = 0.42$, $p < 0.01$; and *SFRP1*, $r = 0.37$, $p = 0.03$; Fig. 2*b*), as was the case in CRC. No correlation was observed between the methylation levels of the three genes in the distal normal-appearing mucosae (Fig. 2*b*). Spearman's correlation coefficients calculated to evaluate the correlations yielded identical results (data not shown).

Interestingly, we found simultaneous methylation of the three target genes, *RASSF1A*, *SFRP1* and *MGMT*, affected the patient prognosis (Supporting Information Fig. 2). These data suggest that DNA methylation in multiple loci is simultaneously accumulated with aging in the distal normal-appearing mucosae in CRC cases and have the clinical impact for CRCs.

DNA methylation in normal-appearing mucosa as a clonal event

Densely methylated CpG promoters result in stable gene silencing.[24] To determine whether low or moderate overall levels of methylation in normal-appearing mucosae represent dense methylation in a subset of the cells in the sample or scattered methylation of different CpGs in a majority of the cells, we performed bisulfite sequences in *RASSF1A* and *MGMT* (Supporting Information Fig. 3). Several clones showed dense methylation in the *RASSF1A* and *MGMT* CpG promoter regions, implying that even in normal-appearing mucosa, a subset of cells harbors dense hypermethylation of tumor suppressor genes, which may be a component of a field defect.[13]

LINE-1 methylation status in CRCs and colon polyps

Aberrant global hypomethylation and regional promoter hypermethylation have been observed in many human malignancies[4]; however, it has not been well documented in non-cancerous tissues. To assess global DNA methylation in

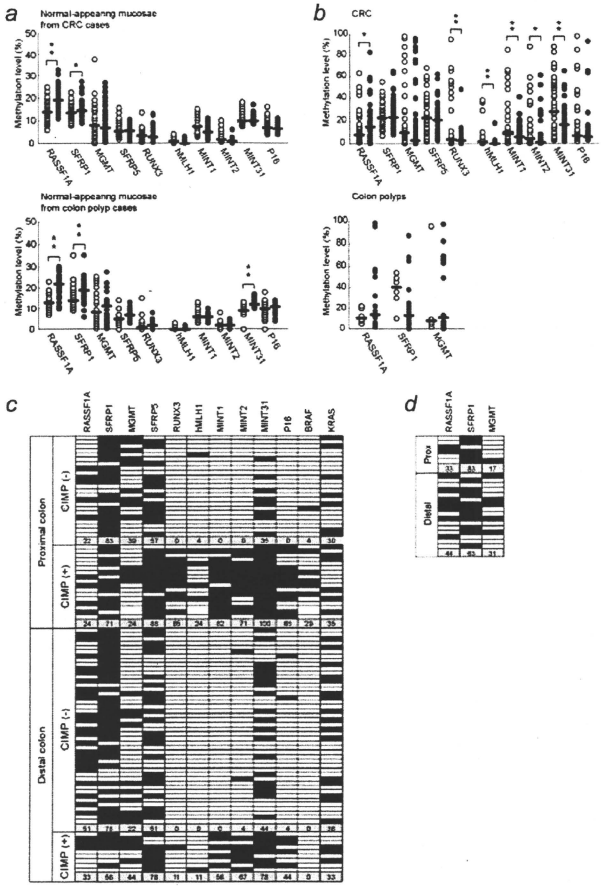

Figure 1. Distribution of the promoter methylation status of 10 genes (*RASSF1A*, *SFRP1*, *MGMT*, *SFRP5*, *RUNX3*, *hMLH1*, *MINT1*, *2* and *31* and *p16*). Levels of methylation measured by bisulfite pyrosequencing methylation analysis in CRC and colon polyp patients. Each circle represents the methylation level of normal-appearing mucosae from CRC or colon polyp cases (*a*) and cancerous tissues from CRC or colon polyp (*b*) from the proximal (white) or distal colon (black). Y-axis indicates the level of methylation of each gene. Horizontal bars denote median methylation levels for each group. $*p < 0.05$; $**p < 0.01$. Methylation frequencies and mutation status of CRC (*c*) and colon polyp patients (*d*). Each column represents the methylation status or *BRAF* or *KRAS* mutations in cancerous tissues or polyps. Black boxes denote methylation levels >15% (methylation positive) or mutations in the *KRAS* or *BRAF* genes. Numbers inside the gray boxes indicate the percentage of cases in which the gene was methylated or mutated. Samples with methylation of at least two of the five CIMP markers (*hMLH1*, *MINT1*, *2*, and *31* and *p16*) were considered CIMP positive. The proximal colon includes the cecum, ascending and transverse colon; the distal includes the descending and sigmoid colon, and the rectum.

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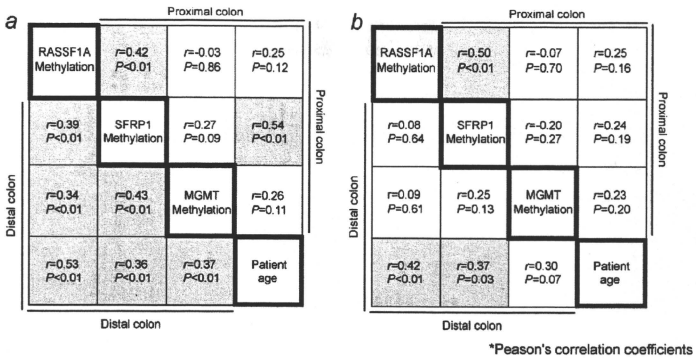

Figure 2. Correlation analysis between methylation levels of three genes (*MGMT*, *RASSF1A* and *SFRP1*) and patient age in the normal-appearing mucosae from CRC (*a*) and colon polyp patients (*b*). Upper right boxes indicate correlations for proximal colon samples, and lower left boxes indicate correlations for distal colon samples; *r* indicates Pearson's correlation coefficients. Colored boxes indicate a significant correlation ($p < 0.01$ or $p < 0.05$).

normal-appearing muocosa, *LINE-1* methylation, a good indicator of global methylation, was assessed by pyrosequencing (Fig. 3).[25,26] Significant hypomethylation was found in both CRCs and colon polyps compared with normal-appearing mucosae (Fig. 3*a*, $p < 0.01$). In CRCs, *LINE-1* methylation was significantly lower in the CIMP-negative than the CIMP-positive group (mean, 56.8%; 95% CI, 54.7–58.9 *vs.* mean, 60.9%; 95% CI, 56.9–65.0, $p < 0.05$). These are concordant with a previous large-scale study using the same technology.[26] Interestingly, correlation coefficient analysis revealed a clear inverse correlation between methylation levels and age in the distal normal-appearing mucosae ($r = -0.48$, $p < 0.01$; Fig. 3*b*). However, no such correlation was observed in the proximal normal-appearing mucosae of either CRC or colon polyp cases (Figs. 3*b* and 3*c*). Along with these findings, normal-appearing mucosae with two or more hypermethylated genes showed a significantly lower level of *LINE-1* methylation than mucosae with one or no hypermethylated genes (mean, 67.0%; 95% CI, 65.4–68.6 *vs.* mean, 69.1%; 95% CI, 67.7–70.3, $p < 0.05$, Fig. 3*d*). These data suggest that in a subset of distal CRC cases, the normal-appearing mucosa becomes susceptible to age-related methylation, wherein even global DNA methylation is affected, and regional hypermethylation and global hypomethylation occur simultaneously in the same individuals.

Genome-wide methylation analysis of CIMP-negative and CIMP-positive CRCs

To decipher the global DNA methylation targets of CIMP-negative cancers, especially of distal CRCs, we performed MCAM in 18 CRCs: 7 CIMP-positive proximal CRCs and 11 CIMP-negative distal CRCs (Materials and methods, and Supporting Information Table 2). We previously reported that a signal ratio of Cy5/Cy3 in excess of 2.0 in MCAM is concordant with hypermethylation status in pyrosequencing analysis.[16] In this study, we validated the MCAM data (Cy5/Cy3>2.0 as methylation positive) with pyrosequencing assays and found that the specificity and sensitivity were both 77% (Supporting Information Table 4). Unsupervised hierarchical clustering analysis using 6,157 genes showed that CIMP-positive proximal CRCs had a prominent cluster of hypermethylated genes, confirming the five classical CIMP markers as reliable predictive markers for CIMP (Fig. 4*a*). Consistently, larger numbers of hypermethylated genes were observed in CIMP-positive proximal CRCs than in CIMP-negative distal CRCs (average of 1,321 genes *vs.* 1,112 genes, $p < 0.05$, Fig. 4*b*).

Although CIMP-positive proximal CRCs were classified by the five classical CIMP markers, four new CIMP markers, *RUNX3*, *CACNA1G*, *NEUROG1* and *CRABP1*, were also highly positive in these CRCs (Fig. 4*c*).[23,27] However, another two new CIMP markers, *SOCS1* and *IGF2*, were not more predictive than the five classical CIMP markers. We noted that mutations in the *BRAF* gene were found in CIMP-positive proximal CRCs, whereas mutations in the *p53* gene appeared in CIMP-negative distal CRCs.

Among the 1,224 genes identified from MCAM data that were methylated in more than half the CRC cases (*i.e.*, in more than 4/7 of the CIMP-positive proximal CRCs and 6/11 of the CIMP-negative distal CRCs), more genes were

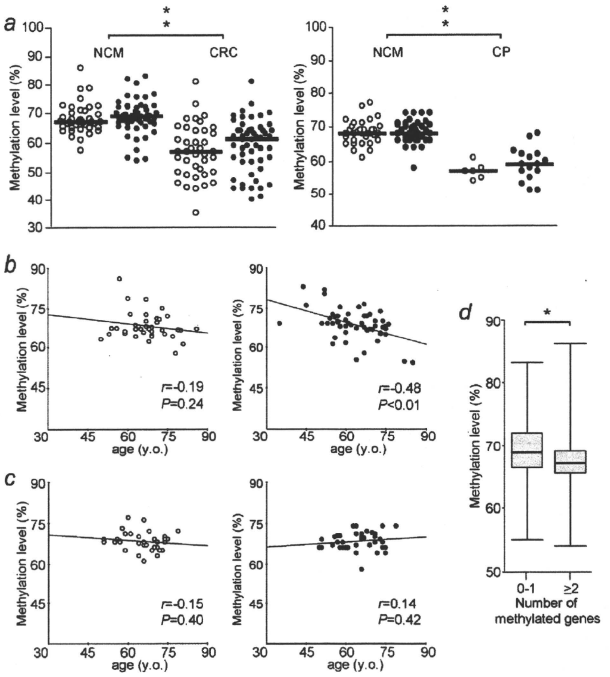

Figure 3. Distribution of *LINE-1* promoter methylation levels measured by bisulfite pyrosequencing methylation analysis in CRC and colon polyp patients. (*a*) Each circle represents the methylation level of normal-appearing mucosa, CRC and colon polyp from the proximal (white) and distal colon (black). Horizontal lines represent median methylation levels for each group. Scatter plots of *LINE-1* methylation level *vs.* patient age in normal-appearing mucosa from CRC (*b*) and colon polyp patients (*c*) taken from the proximal (white circles) or distal colon (black circles). (*d*) Box-and-whisker plot of *LINE-1* methylation level in normal-appearing mucosae with hypermethylation detected in 0–1 or ≥2 of 3 genes, *RASSF1A*, *SFRP1* and *MGMT*. The mean is marked by a bold line inside the box whose ends denote the upper and lower quartiles. Error bars represent 5 and 95 percentile values. $*p < 0.05$; $**p < 0.01$.

methylated in CIMP-positive proximal CRC than CIMP-negative distal CRCs (1,056 genes *vs.* 504 genes, among which 336 genes were commonly methylated in both groups, $p < 0.01$, Fig. 5*a*). This tendency was also true when analyzing genes methylated in at least 30% of CRC cases ($p < 0.01$). Although CIMP-positive proximal CRCs appear to be more robustly affected by DNA methylation, there is a subset of genes preferentially methylated in CIMP-negative distal CRCs.

Combined array data analysis of CRCs and eight normal-appearing mucosae revealed that DNA methylation in either both normal-appearing mucosae and CRCs (age-related methylation, type A genes), or specifically in CRCs (cancer-specific methylation, type C genes).[5] The ratios of type A to type C genes in CIMP-negative distal CRCs and CIMP-positive proximal CRCs were significantly different. More than half of the hypermethylated genes in CIMP-negative distal CRCs were also methylated in the normal-appearing mucosae, as opposed to CIMP-positive proximal CRCs, where more than 60% of hypermethylated genes were cancer specific ($p < 0.01$, Fig. 5*b*). A heat-map overview of 168 genes (type C, 79 genes; type A, 89 genes), which were methylated in more than half of CIMP-negative distal CRCs revealed that DNA methylation was also found in CIMP-positive

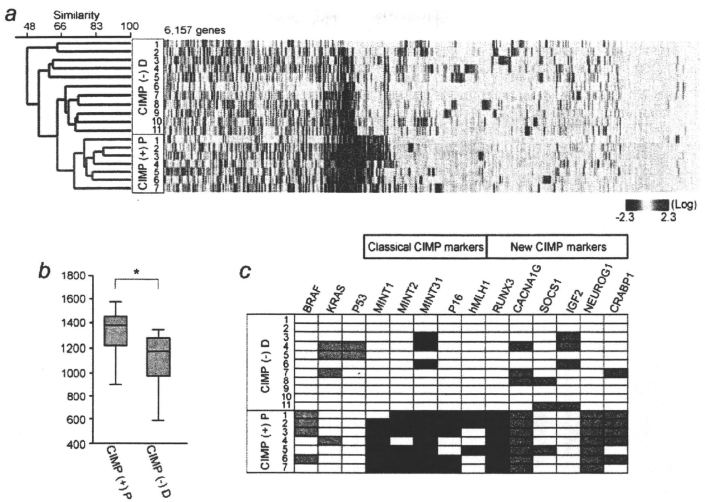

Figure 4. MCAM analysis of CRC cases. (*a*) Heat-map overview and cluster analysis of hierarchical cluster analysis using the DNA methylation status of 18 samples [11 CIMP-negative distal CRCs (CIMP (–) D), and 7 CIMP-positive proximal CRCs (CIMP (+) P)]. Red, yellow and blue in the cells reflect methylation levels as indicated in the scale bar below the matrix (log2-transformed scale). All 7 CIMP-positive CRCs fall into one subgroup (blue line). (*b*) Box-and-whisker plot showing the number of methylated genes in CIMP-positive proximal CRCs and CIMP-negative distal CRCs. The median is marked by a bold line inside the box whose ends represent the upper and lower quartiles. Error bars denote 5 and 95 percentile values. $*p < 0.05$. (*c*) Comparison of methylation status of CIMP-positive and CIMP-negative CRCs. Each column represents the mutation or methylation status of each CRC case. Blue boxes indicate mutations in the *KRAS*, *BRAF* or *p53* genes. Black and red boxes indicate a methylation-positive status determined by pyrosequencing or MCAM analysis (average Cy3/Cy5 signal ratio > 2.0), respectively.

proximal CRCs in a certain extend (Fig. 5*c*). We further validated the identified hypermethylated genes in CIMP-negative distal CRCs. Methylation levels of *HOXA5* (type A gene) and *PDE10A* (type C gene) were examined by pyrosequencing in CRCs and their corresponding normal-appearing mucosae (Fig. 5*d*). In the normal-appearing mucosae of CRC cases, DNA methylation levels of both genes were significantly higher in the distal than the proximal colon (HOXA5, $p < 0.05$; PDE10A, $p < 0.01$). DNA methylation levels of *PDE10A* genes were also higher in the distal than the proximal CRCs ($p < 0.01$).

Discussion

Alterations in DNA methylation represent epigenetic phenomena that appear to be early events in tumorigenesis.[28] Recent comprehensive studies have suggested that CIMP is a distinct colon tumorigenesis pathway that shows an accumulation of high rates of aberrant promoter methylation events. CIMP tumors have a characteristic phenotype with such features as *BRAF* or *KRAS* mutations, a specific histology (mucinous or poorly differentiated) and proximal location.[3,6,27,29–31] In addition, cancer-specific DNA methylation is more frequent than age-related DNA methylation in a subclass of CIMP-positive CRCs.[6] The CIMP-positive tumors we analyzed exhibited these features; however, we also found frequent methylation of some genes in the CIMP-negative distal CRCs. This suggests that during colon tumorigenesis, other mechanisms besides CIMP could be causing aberrant DNA methylation in the distal colon.

By focusing on the differences in hypermethylated genes between CIMP-positive proximal and CIMP-negative distal CRCs, our genome-wide analysis revealed that hypermethylated genes in the CIMP-negative distal CRCs largely overlapped with those in the CIMP-positive proximal CRCs; however, a set of genes was preferentially methylated in the CIMP-negative distal CRCs. Along with *RASSF1A* and *SFRP1*, two identified genes, *HOXA5* and *PDE10A*, were also frequently methylated in distal normal-appearing mucosae

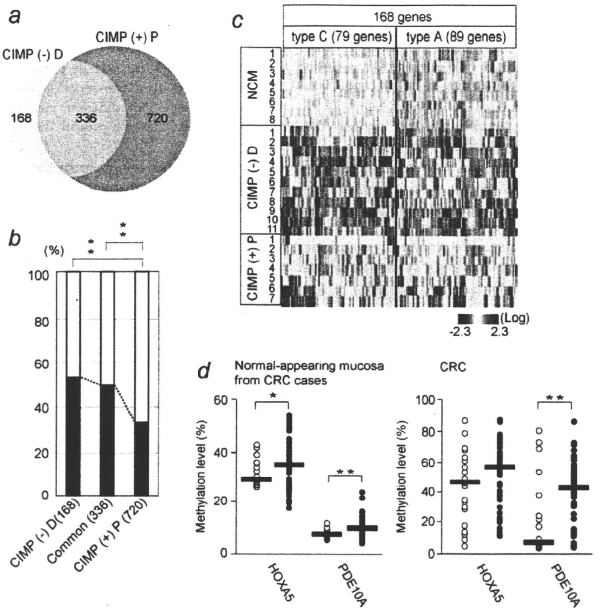

Figure 5. MCAM and pyrosequencing analysis associated with CIMP-negative distal CRCs. (*a*) Venn diagram illustrating the number of genes that were methylation positive in more than half of CRC cases as determined by MCAM. More genes were methylated in CIMP-positive proximal CRCs than CIMP-negative distal CRCs (1,056 genes *vs.* 504 genes, among which 336 genes were commonly methylated in both groups). (*b*) Percentages of type A (black box) and type C (white box) genes among CIMP-negative distal (CIMP (–) D), CIMP-positive proximal (CIMP (+) P) CRCs and both (common). $^{**}p < 0.01$. (*c*) Heat-map overview with 168 genes which were methylation positive in more than half of CIMP-negative distal CRC cases. Red, yellow and blue in the cells reflect methylation levels as indicated in the scale bar below the matrix (log2-transformed scale). (*d*) Levels of methylation in *HOXA5* and *PDE10A* measured by bisulfite pyrosequencing methylation analysis in CRC cases. Each circle represents the methylation levels of normal-appearing mucosae (*b*) and CRCs (*c*) from the proximal (white) or distal colon (black). $^{*}p < 0.05$; $^{**}p < 0.01$.

and CRCs. HOXA5, a developmental regulator of several tissues, has also been known to act as a tumor suppressor through induction of apoptosis of cancer cells.[32–34] A homozygous deletion at 6q26-27, which includes the *PDE10A* genes, has been observed in glioblastoma.[35] Simultaneous silencing of these sets of genes by epigenetic mechanisms in addition to genetic alteration (*e.g.*, mutations in the *p53* gene) may contribute the distal colon tumorigenesis.

The multistep carcinogenesis of CRCs has suggested the existence of a period of preneoplastic condition, field defect, in which cells accumulate genetic and epigenetic alterations and are predisposed to tumor development.[2,36,37] Age-related epigenetic defects have been proposed as potential sources of the field defect in colon carcinogenesis.[13,38] In this study, we analyzed the DNA methylation status of normal-appearing mucosae to elicit the DNA methylation behavior of distal CRC compared with proximal CRC. In the distal normal-appearing mucosae of CRCs, methylation levels of *RASSF1A*, *SFRP1* and *MGMT* were significantly correlated with one another and associated with age, whereas global methylation levels (estimated as *LINE-1* methylation levels) diminished with age. Both regional hypermethylation and global hypomethylation appear to occur simultaneously in a subset of distal normal-appearing mucosae. These data indicated that distal CRCs might be closely associated by field defect where age-related DNA methylation target genes were embedded,

involving the pathways and providing the cell with selective advantages that promote tumor progression.

The characteristic DNA methylation behavior in distal normal-appearing mucosa and CRCs may be partially explained by various environmental factors in the distal colon, where continuous exposure to stool is common.[9,10,39–42] In addition, absorption of water from the stool increases the risk of exposure to higher concentrations of exogenous substances that may act as epimutagenes, proposed environmental factors that can affect the epigenetic status of genes.[30,42] The levels of exposure to environmental factors, patterns of genetic defects and types of epimutagenes present differ in each case by location and may affect epigenetic variations. Consequently, accumulation patterns of DNA methylation in normal-appearing mucosae is not uniform by location, as the present study also indicates.

Although the fundamental cause of aberrant DNA methylation in cancers is still under investigation, combinations of such environmental exposures and genetic alterations might facilitate the deregulation of epigenetic control. In cancer cell lines, hypermethylation is triggered by low and random levels of DNA methylation (seeding) together with gene inactivation by the removal of Sp1 transcription factor binding sites.[43] An Sp1/Sp3 binding polymorphism in the *RIL* promoter has also been reported to confer methylation protection.[44] Dysregulation of such *cis*-acting factors in addition to environmental exposures may be pivotal in perpetuating the hypermethylation of a subset of genes. Nevertheless, our study showed here that different mechanisms of acquiring epigenetic changes may be present during the tumorigenesis of CRCs.

In conclusion, our comprehensive analysis deciphered that particular patterns of aberrant DNA methylation in CIMP-negative distal CRCs are active during colon tumorigenesis; global DNA methylation levels were decreased and age-related DNA methylation in multiple genes was inappropriately induced, which may dictate a characteristic pathogenesis. Because recent combined genetic and epigenetic analyses of sporadic CRC suggest that there are different subsets possessing distinct clinicopathological features,[45] elucidation of the precise roles of epigenetic abnormalities might be a great help for the prevention, screening and treatment of CRCs.

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Irinotecan plus S-1 (IRIS) versus fluorouracil and folinic acid plus irinotecan (FOLFIRI) as second-line chemotherapy for metastatic colorectal cancer: a randomised phase 2/3 non-inferiority study (FIRIS study)

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Summary

Background **Fluorouracil and folinic acid with either oxaliplatin (FOLFOX) or irinotecan (FOLFIRI) are widely used as first-line or second-line chemotherapy for metastatic colorectal cancer. However, infusional fluorouracil-based regimens, requiring continuous infusion and implantation of an intravenous port system, are inconvenient. We therefore planned an open-label randomised controlled trial to verify the non-inferiority of irinotecan plus oral S-1 (a combination of tegafur, 5-chloro-2,4-dihydroxypyridine, and potassium oxonate; IRIS) to FOLFIRI as second-line chemotherapy for metastatic colorectal cancer.**

Methods **Between Jan 30, 2006, and Jan 29, 2008, 426 patients with metastatic colorectal cancer needing second-line chemotherapy from 40 institutions in Japan were randomly assigned by a computer-based minimisation method to receive either FOLFIRI (n=213) or IRIS (n=213). In the FOLFIRI group, patients received folinic acid (200 mg/m²) and irinotecan (150 mg/m²) and then a bolus injection of fluorouracil (400 mg/m²) on day 1 and a continuous infusion of fluorouracil (2400 mg/m²) over 46 h, repeated every 2 weeks. In the IRIS group, patients received irinotecan (125 mg/m²) on days 1 and 15 and S-1 (40–60 mg according to body surface area) twice daily for 2 weeks, repeated every 4 weeks. The primary endpoint was progression-free survival, with a non-inferiority margin of 1·333. Statistical analysis was on the basis of initially randomised participants. This study is registered with ClinicalTrials.gov, number NCT00284258.**

Findings **All randomised patients were included in the primary analysis. After a median follow-up of 12·9 months (IQR 11·5–18·2), median progression-free survival was 5·1 months in the FOLFIRI group and 5·8 months in the IRIS group (hazard ratio 1·077, 95% CI 0·879–1·319, non-inferiority test p=0·039). The most common grade three or four adverse drug reactions were neutropenia (110 [52·1%] of 211 patients in the FOLFIRI group and 76 [36·2%] of 210 patients in the IRIS group; p=0·0012), leucopenia (33 [15·6%] in the FOLFIRI group and 38 [18·1%] in the IRIS group; p=0·5178), and diarrhoea (ten [4·7%] in the FOLFIRI group and 43 [20·5%] in the IRIS group; p<0·0001). One treatment-related death from hypotension due to shock was reported in the FOLFIRI group within 28 days after the end of treatment; no treatment-related deaths were reported in the IRIS group.**

Interpretation **Progression-free survival with IRIS is not inferior to that with FOLFIRI in patients receiving second-line chemotherapy for metastatic colorectal cancer. Treatment with IRIS could be an additional therapeutic option for second-line chemotherapy in metastatic colorectal cancer.**

Funding **Taiho Pharmaceutical Co Ltd and Daiichi Sankyo Co Ltd.**

Introduction

The combination of fluorouracil and folinic acid with either oxaliplatin (FOLFOX) or irinotecan (FOLFIRI) has been established as the standard first-line chemotherapy for metastatic colorectal cancer.[1] For second-line chemotherapy for patients resistant to fluorouracil, randomised comparative studies have shown that irinotecan monotherapy was effective.[2,3] Rougier and colleagues[4] showed comparable efficacy of FOLFIRI, FOLFOX, and irinotecan and oxaliplatin (IROX) in patients unresponsive to fluorouracil in a randomised phase 2 study.

Tournigand and colleagues[5] showed that, in patients with metastatic colorectal cancer who were randomly assigned to receive FOLFIRI or FOLFOX as first-line chemotherapy and then crossed over to receive the other as second-line chemotherapy, overall survival was similar in both groups. Consequently, initial treatment with FOLFOX and then second-line treatment with FOLFIRI or vice versa is recommended as standard therapy.[6] However, infusional fluorouracil-based regimens, requiring continuous infusion and implantation of an intravenous port system, are inconvenient and sometimes associated with catheter-related problems such as infection and thrombosis.

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See Reflection and Reaction page 808

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S-1 is an oral fluoropyrimidine consisting of tegafur, 5-chloro-2,4-dihydroxypyridine (CDHP), and potassium oxonate, in which tegafur is a pro-drug of fluorouracil, CDHP is a dihydropyrimidine dehydrogenase (DPD) inhibitor maintaining the serum concentration of fluorouracil, and potassium oxonate is an inhibitor of orotate phosphoribosyl transferase, reducing gastrointestinal toxicities. Response rates for monotherapy with S-1 are around 35% for colorectal cancer, and it is suggested that DPD inhibition in tumour cells might contribute to antitumour effects because S-1 has been effective against many solid tumours with high DPD expression.[7] Clinically, responses rates of 52·5–62·5% have been reported in phase 2 studies of irinotecan plus S-1 combination therapy, with median progression-free survival of 7·8–8·6 months for first-line treatment for metastatic colorectal cancer.[8–10] These results suggest that the efficacy of IRIS might be comparable to that of FOLFIRI and that IRIS might also be more convenient for both patients and medical facilities.

We did a phase 2/3 randomised study (FIRIS study) to verify the non-inferiority of IRIS to FOLFIRI in patients with metastatic colorectal cancer in whom first-line chemotherapy failed.

Methods

Patients

Inclusion criteria were histologically confirmed colorectal adenocarcinoma; unresectable metastatic disease; age 20–75 years; Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; withdrawal from first-line chemotherapy due to toxicity or progressive disease, or relapse within 24 weeks after the final dose of preoperative or postoperative chemotherapy; no previous treatment with irinotecan; sufficient oral intake; adequate organ function (white blood cell count 3000–12 000 cells per μL, platelet ≥100 000 per μL, aspartate aminotransferase [AST] ≤100 IU/L, alanine aminotransferase [ALT] ≤100 IU/L, total bilirubin ≤25·7 μmol/L [≤15 mg/L], and creatinine ≤106·1 μmol/L [≤12 mg/L]); and no abnormal electrocardiographic findings within 28 days before enrolment. Exclusion criteria were pregnancy or lactation; second non-colorectal cancer; complications such as ileus, uncontrolled diabetes mellitus, or hypertension; severe diarrhoea; clinically evident gastrointestinal haemorrhage; and ascites or pleural effusion needing treatment.

The protocol of this study was approved by the institutional review board or ethics committee of each institution. The study was conducted in compliance with the Declaration of Helsinki. Written informed consent was obtained from all patients participating in the study.

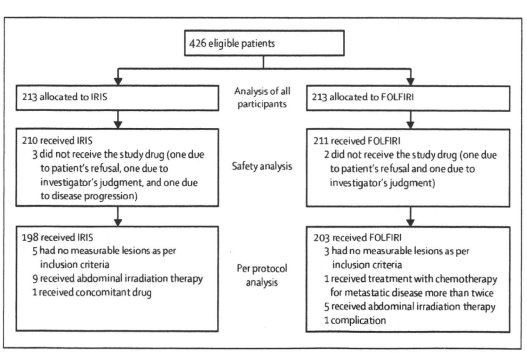

Figure 1: **Trial profile**

Randomisation and masking

Investigators provided the patient's details to the central registration centre through a web-based registration system. After an eligibility check, patients were randomly assigned to receive FOLFIRI or IRIS at the central registration centre by a computer program, by use of a minimisation method with stratification by institution, prior therapy (with or without oxaliplatin), and performance status (0 or 1). Assignment of patients was concealed from the investigator. Treatment assignment was not masked from the investigators or patients.

Procedures

Our randomised, open-label, phase 2/3 study in patients with the second-line metastatic colorectal cancer was done in 40 institutions in Japan (mainly hospitals and medical centres). In the phase 2 portion, safety was assessed in patients treated with either FOLFIRI (30 patients) or IRIS (30). Additionally, the response rate in the first 50 patients in the IRIS group was assessed because IRIS is an unfamiliar regimen in Japan. An independent data and safety monitoring board reviewed our results (safety and efficacy in the phase 2 portion; safety in the phase 3 portion), and approved proceeding to the phase 3 portion. The final analysis was done by use of the combined data from phase 2 and 3 portions.

Patients in the FOLFIRI group received concurrent folinic acid (200 mg/m²) and irinotecan (150 mg/m²) and then a bolus injection of fluorouracil (400 mg/m²) on day 1 and subsequent continuous infusion of fluorouracil (2400 mg/m²) over 46 h, repeated every 2 weeks (4 weeks counted as one cycle). In the FOLFIRI group, the dose of irinotecan was 150 mg/m², the approved dose in Japan.[11] The IRIS group received irinotecan (125 mg/m²) on days 1 and 15 and S-1 (40 mg for patients with body surface area [BSA] <1·25 m²; 50 mg for patients with BSA 1·25<1·5 m²; 60 mg for patients with BSA ≥1·5 m²) twice daily for 2 weeks from days 1–14 and then a 2-week pause, on the basis of results of phase 2 studies.[12,13] We selected this regimen from several documented regimens of irinotecan and S-1 to match the regimen of FOLFIRI in the control arm. Regimens in which irinotecan is given every 2 weeks[12,13] and every 3 weeks are in clinical use in Japan.[8]

In both FOLFIRI and IRIS groups, treatment was delayed until recovery if white blood cell count fell below than 3000 cells per μL, platelets fell below 100000 per μL, AST or ALT were over 100 IU/L, total bilirubin was higher than 25·7 μmol/L, creatinine was higher than 106·1 μmol/L, the patient experienced diarrhoea of grade one or greater, or other non-haematological toxicities greater than grade two. If a patient experienced a grade four haematological or grade three or higher non-haematological toxicity, the dose was decreased by one level for the next course of treatment, and therapy was resumed.

Treatment was continued until progressive disease, unacceptable toxicity, or patient's refusal to continue treatment. Because molecularly targeted agents such as bevacizumab, cetuximab, and panitumumab were not approved in Japan at the start of our study, no restriction for such agents was specifically placed on treatment before or after the study.

Physical examination, electrocardiography, performance status, and laboratory tests were done at baseline and repeated at least every 2 weeks during treatment. Tumours were assessed at baseline (within 1 month before enrolment), and at 2, 3, and 4 months after enrolment, and thereafter every 2 months until progression. Progression was defined as progressive disease on the basis of the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0, clinical progression judged by the investigator, or death from any cause without progression.

Progression-free survival was counted from the date of randomisation to the date when the progressive disease was first confirmed by the investigator's assessment. For patients without documented progressive disease, data was censored on the date of the last tumour assessment with non-progression status. Overall survival was calculated from the date of randomisation to the date of death or confirmation of survival.

Toxicity was evaluated on the basis of the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

Statistical analysis

The primary efficacy analysis was done with all randomised patients; we also did a per-protocol analysis in which patients in whom there was a major violation such as inclusion or exclusion criteria or protocol treatments were excluded. Safety was assessed in all patients who received at least one dose of the study drug.

The primary objective of our study was to show non-inferiority of IRIS to FOLFIRI for progression-free survival in the whole randomised population. On the basis of data from previous reports in patients with metastatic colorectal cancer who received second-line chemotherapy, median progression-free survival with both FOLFIRI and IRIS was assumed to be 4 months. The steering committee deemed that response assessment could not be repeated more frequently than once a month, so a difference in progression-free survival shorter than 1 month could not be detected precisely. Thus, progression-free survival with IRIS that was 1 month shorter than with FOLFIRI would be acceptable as a lower margin for inferiority, given the expected hazard ratio [HR] of 1·0. The 95% CI upper limit of the HR, calculated using Cox regression analysis with stratification factors other than institution, was prespecified as less than 1·333, meaning the null hypothesis was that median progression-free survival with IRIS would be 1 month shorter than with FOLFIRI. Because 379 events were needed to show non-inferiority with a two-sided α of 0·05 and a power of 80%, a target sample size of 400 patients was required.

Secondary endpoints were overall survival, response rate, and toxicity. Subgroup analyses were done to establish whether therapeutic efficacy was affected by sex, age, histological type, performance status, and prior chemotherapy with or without oxaliplatin. Progression-free and overall survival were estimated using the Kaplan-Meier method. The 95% CI for median progression-free and overall survival was calculated using the method of Brookmeyer and Crowley.[14] All p values were two-sided. All statistical analyses were done with SAS version 8.2. This study is registered with ClinicalTrials.gov, number NCT00284258.

Role of the funding source

The funding source had no role in the study design, data collection, data analysis, or interpretation. All authors had access to all of the data. The corresponding author had final responsibility for decision to submit for publication.

	FOLFIRI (n=213)	IRIS (n=213)
Sex		
Male	123 (57·7%)	120 (56·3%)
Female	90 (42·3%)	93 (43·7%)
Age (years)	63·0 (32–75)	61·0 (29–75)
ECOG performance status		
0	160 (75·1%)	158 (74·2%)
1	53 (24·9%)	55 (25·8%)
Histological type		
Well differentiated	62 (29·1%)	60 (28·2%)
Moderately differentiated	124 (58·2%)	133 (62·4%)
Poorly differentiated	13 (6·1%)	8 (3·8%)
Other	13 (6·1%)	11 (5·2%)
Undetermined	1 (0·5%)	1 (0·5%)
Previous chemotherapy with oxaliplatin		
Yes	128 (60·1%)	129 (60·6%)
No	85 (39·9%)	84 (39·4%)
Number of metastatic sites		
One	92 (43·2%)	88 (41·3%)
Two or more	120 (56·3%)	124 (58·2%)

Data are number (%) or median (range). FOLFIRI=folinic acid, fluorouracil, and irinotecan. IRIS=irinotecan and S-1. ECOG=Eastern Cooperative Oncology Group.

Table 1: **Baseline patient characteristics**

Results

426 patients from 40 institutions in Japan were enrolled in the study from Jan 30, 2006, to Jan 29, 2008, and randomised either to the FOLFIRI or IRIS group (213 patients in each; figure 1). Of the per-protocol population, 203 patients were in the FOLFIRI group and 198 were in the IRIS group; reasons for exclusion are shown in figure 1. All patients who received study treatment (211 patients in the FOLFIRI group and 210 patients in the IRIS group) were included in the safety evaluation. Baseline characteristics were well balanced between the two groups (table 1).

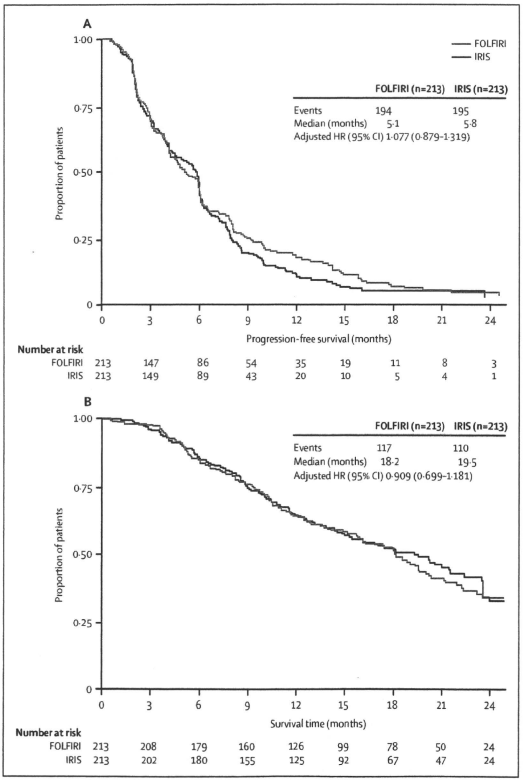

Figure 2: **Progression-free survival (A) and overall survival (B)**
FOLFIRI=infusional fluorouracil, folinic acid, and irinotecan. IRIS=irinotecan plus S-1. HR=hazard ratio.

The mean number of cycles of protocol treatment was 4·7 (range 1–20) for FOLFIRI and 4·9 (1–23) for IRIS. Median relative dose intensities to the planned dose were almost identical: irinotecan 78·3%, bolus fluorouracil 76·9%, and infusional fluorouracil 81·5% in the FOLFIRI group, and irinotecan 78·3% and S-1 88·9% in the IRIS group. Treatments were discontinued because of disease progression in 68·5% (146 patients) in the FOLFIRI group and in 66·2% (141) in the IRIS group, adverse events in 10·8% (23) and in 16·9% (36), and patient's refusal 1·9% (four) and 6·1% (13). 179 patients in the FOLFIRI group and 184 patients in the IRIS group needed a dose delay or dose reduction. Treatment after the trial (ie, treatment after failure of second-line regimen) was given to 159 (74·6%) patients in the FOLFIRI group and 147 (69·0%) in the IRIS group. As third-line treatment, an oxaliplatin-containing regimen was given to 58 (27·2%) patients in the FOLFIRI and 63 (29·6%) in the IRIS group. Molecularly targeted agents as treatments after the trial were used in 24 patients in the FOLFIRI group and 16 in the IRIS group.

As of Dec 31, 2008, collection of progression-free and overall survival data was cut off, with 389 confirmed events (194 FOLFIRI and 195 IRIS). Median follow-up was 12·9 months (IQR 11·5–18·2). Median progression-free survival was 5·1 months in the FOLFIRI group and 5·8 months in the IRIS group. In the entire randomised population, the HR of progression-free survival in the IRIS group compared with the FOLFIRI group was 1·077 (95% CI 0·879–1·319, p=0·039). Similar results were seen in the per protocol population: median progression-free survival was 5·1 months in the FOLFIRI group and 5·7 in the IRIS group (HR 1·050, 95% CI 0·851–1·294).

The data on overall survival are preliminary because of short follow-up. 117 of the 213 patients in the FOLFIRI group and 110 of the 213 patients in the IRIS group died due to any cause. Median overall survival in the entire randomised population was 18·2 months in the FOLFIRI group and 19·5 months in the IRIS group (HR 0·909, 95% CI 0·699–1·181; figure 2). In the per protocol population, median overall survival was 18·1 months in the FOLFIRI group and 19·3 months in IRIS group (HR 0·896, 95% CI 0·685–1·172).

The overall response rate was 16·7% (one patient had a complete response, 28 patients had a partial response) of 174 patients with evaluable response data in the FOLFIRI group and 18·8% (one patient had a complete response, 33 patients had a partial response) of 181 in the IRIS group.

Figure 3 shows the results of subgroup analyses of progression-free survival. Although no interaction was identified between sex, age, histological type, or performance status and therapeutic effects of IRIS compared with FOLFIRI, a statistically significant interaction was noted between prior chemotherapy (with or without oxaliplatin) and therapeutic effects (p=0·030). In the subgroup of patients receiving prior chemotherapy with oxaliplatin, median progression-free survival was

5·7 months in the IRIS group and 3·9 months in the FOLFIRI group (adjusted HR 0·876, 95% CI 0·677–1·133), whereas in patients without prior oxaliplatin treatment it was 6·0 months and 7·8 months, respectively (HR 1·490, 95% CI 1·079–2·059). A similar tendency was noted in the overall survival (figure 4).

Table 2 lists major adverse events. In the two groups, the incidences of adverse events were not markedly different from those previously reported, and none of the adverse events were unexpected. Significantly more patients in the FOLFIRI group experienced grade three or four neutropenia than did those in the IRIS group (110 [52·1%] of 211 patients in the FOLFIRI group *vs* 76 [36·2%] of 210 in the IRIS group; p=0·0012); 33 (15·6%) of patients in the FOLFIRI group and 38 (18·1%) in the IRIS group experienced leucopenia (p=0·5178). The most common non-haematological toxicities were diarrhoea (10 [4·7%] in the FOLFIRI group *vs* 43 [20·5%] in the IRIS group; p<0·0001), anorexia (11 [5·2%] *vs* 23 [11·0%]; p=0·0329), nausea (nine [4·3%] *vs* four [1·9%]; p=0·2593), fatigue (seven [3·3%] *vs* 18 [8·6%]; p=0·0242), and febrile neutropenia (two [0·9%] *vs* 10 [4·8%]; p=0·0205), all at grade three (table 2). One treatment-related death from hypotension due to shock was reported in the FOLFIRI group within 28 days after the end of treatment; no treatment-related deaths were reported in the IRIS group.

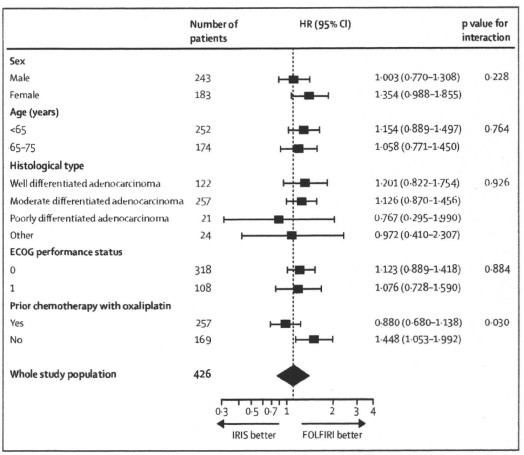

Figure 3: **Subgroup analysis of progression-free survival**
HR=hazard ratio.

Discussion

Our randomised study, comparing FOLFIRI and IRIS as second-line chemotherapy for patients with metastatic colorectal cancer, shows the non-inferiority of IRIS to FOLFIRI. Similar results were obtained in both the entire randomised population and in the more conservative per-protocol analysis. Response rates and overall survival were equivalent between the groups. To our knowledge, this is the first phase 3 trial that shows non-inferiority of oral fluoropyrimidine plus irinotecan therapy to FOLFIRI. From the point of convenience, there has been substantial demand for replacing infusional fluorouracil-based regimens with oral fluorouracil agents. Our study was not designed to collect specific data on working hours of clinicians or the quality of life of patients. However, unlike FOLFIRI, IRIS does not contain infusional fluorouracil and thus does not require a long infusion process, reducing the inconvenience to both patients and clinicians. Additionally, no infuser pump is needed, providing a great advantage to patients. Randomised studies comparing FOLFOX with capecitabine plus oxaliplatin (XELOX) in patients with metastatic colorectal cancer showed that XELOX was non-inferior to FOLFOX.[15,16] By contrast, Fuchs and colleagues[17] reported that progression-free survival with capecitabine plus irinotecan (CapeIRI; 5·8 months) was clearly inferior to that with FOLFIRI (7·6 months) as the first-line chemotherapy for metastatic colorectal cancer, and CapeIRI was associated with a higher incidence of gastrointestinal toxicities and hand-foot syndrome, resulting in discontinuation for reasons other than disease progression.

In our study, the incidence of grade three or worse diarrhoea, fatigue, febrile neutropenia, and anorexia were significantly higher in the IRIS group than the FOLFIRI group. In general, oral fluorouracil-derivative drugs have been shown to be associated with a higher incidence of diarrhoea.[15,17–19] This might also be applicable to S-1. It might be attributable to 2-week treatment with S-1 in IRIS compared with 2-day treatment with fluorouracil in FOLFIRI. However, there was no significant difference in the number of courses or dose intensity between groups. It is thought that all adverse events could be controlled by supportive care, treatment interruptions, or dose reduction, with little effect on treatment continuity. Of note, in the IRIS group, grade four diarrhoea was not detected and fewer of the patients enrolled towards the end of the study experienced grade three diarrhoea.

The incidence of fluorouracil-induced diarrhoea, especially by oral fluoropyrimidines, has been shown to be higher in non-Asian patients than Asian patients.[17,19–21] We speculate that IRIS therapy might also be less feasible in non-Asian patients; therefore, the optimum dose of S-1 in IRIS should be clarified for this population. The reported incidence of hand-foot syndrome due to fluoropyrimidine derivatives containing DPD inhibitors, such as S-1, was low in both Japanese and western trials.[22] In our study, grade three hand-foot syndrome, which is frequently noted with capecitabine-based regimens both in Japanese and non-Asian patients, was not noted in the IRIS group.

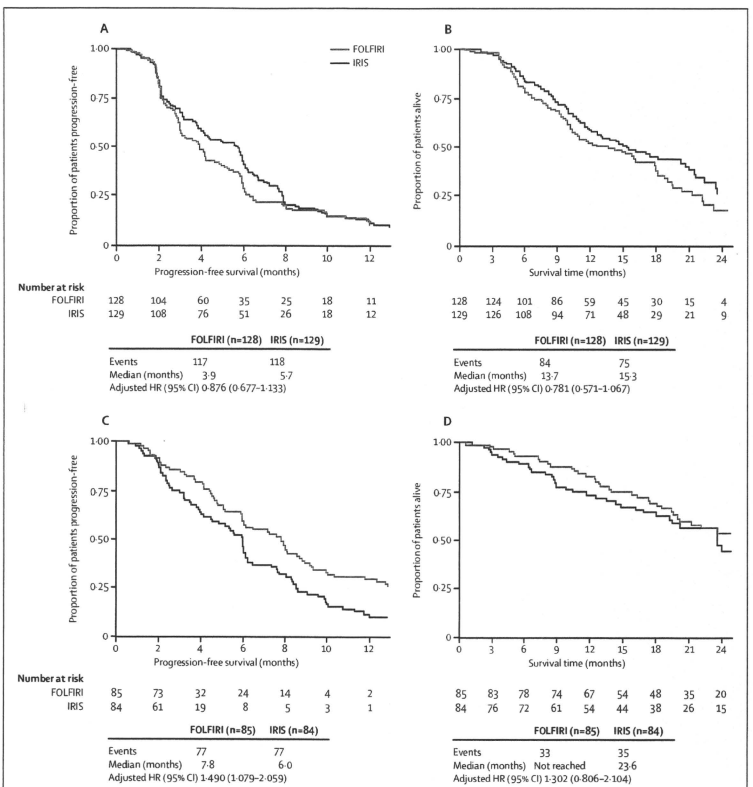

Figure 4: **Survival according to prior chemotherapy**
Progression-free survival with prior oxaliplatin (A). Overall survival with prior oxaliplatin (B). Progression-free survival without prior oxaliplatin (C). Overall survival without prior oxaliplatin (D). FOLFIRI=infusional fluorouracil, folinic acid, and irinotecan. IRIS=irinotecan plus S-1. HR=hazard ratio.

When our trial was started, FOLFOX was already the standard first-line treatment worldwide, but because oxaliplatin had just been launched in Japan, patients who received prior chemotherapy regimens without oxaliplatin were also enrolled. In the subgroup that received prior oxaliplatin, the adjusted HR for progression-free survival of IRIS to FOLFIRI was 0·876 (95% CI 0·677–1·133) suggesting that IRIS was non-inferior to FOLFIRI after failure on oxaliplatin-containing regimens. In this subgroup, the median progression-free survival associated with IRIS was 5·7 months, and much better than the previously reported progression-free survival associated with FOLFIRI in patients who received prior chemotherapy with a fluoropyrimidine and oxaliplatin.[5,23] FOLFOX or FOLFIRI as the first-line chemotherapy and subsequent crossover in the second line is the most common treatment strategy for metastatic colorectal cancer, although there is no evidence of superiority of FOLFIRI over irinotecan alone. In Japan, the approved dose of irinotecan (150 mg/m², every 2 weeks) alone is lower than that in western countries, and monotherapy with irinotecan (350 mg/m², every 3 weeks) could not be used. Both IRIS

	FOLFIRI (n=211)			IRIS (n=210)			p value (grade 3–4)
	All grades	Grade 3	Grade 4	All grades	Grade 3	Grade 4	
Neutropenia	179 (84·8%)	76 (36·0%)	34 (16·1%)	139 (66·2%)	54 (25·7%)	22 (10·5%)	0·0012
Leucopenia	170 (80·6%)	32 (15·2%)	1 (0·5%)	154 (73·3%)	32 (15·2%)	6 (2·9%)	0·5178
Anaemia	115 (54·5%)	13 (6·2%)	1 (0·5%)	156 (74·3%)	19 (9·0%)	2 (1·0%)	0·2221
Thrombocytopenia	63 (29·9%)	1 (0·5%)	1 (0·5%)	74 (35·2%)	0 (0·0%)	0 (0·0%)	0·4988
Diarrhoea	125 (59·2%)	10 (4·7%)	0 (0·0%)	167 (79·5%)	43 (20·5%)	0 (0·0%)	<0·0001
Fatigue	144 (68·2%)	7 (3·3%)	0 (0·0%)	153 (72·9%)	18 (8·6%)	0 (0·0%)	0·0242
Febrile neutropenia	3 (1·4%)	2 (0·9%)	0 (0·0%)	10 (4·8%)	10 (4·8%)	0 (0·0%)	0·0205
Mucositis or stomatitis	92 (43·6%)	1 (0·5%)	0 (0·0%)	102 (48·6%)	6 (2·9%)	0 (0·0%)	0·0677
Anorexia	129 (61·1%)	11 (5·2%)	0 (0·0%)	141 (67·1%)	23 (11·0%)	0 (0·0%)	0·0329
Nausea	111 (52·6%)	9 (4·3%)	0 (0·0%)	99 (47·1%)	4 (1·9%)	0 (0·0%)	0·2593

Data are number (%).

Table 2: **Safety analysis**

and FOLFIRI showed longer median progression-free survival than reported in trials of monotherapy with irinotecan.[3,22] Thus, irinotecan-based regimens, such as FOLFIRI and IRIS, delivered every 2 weeks, should be considered after FOLFOX failure, especially in Japan. By contrast, in the subgroup of patients previously treated without oxaliplatin, progression-free survival was longer in the FOLFIRI group than in the IRIS group (HR 1·490, 95% CI 1·079–2·059). In this subset, prior fluorouracil monotherapy (oral, bolus) had failed in some patients. For these patients, FOLFIRI might have greater efficacy than IRIS. Nonetheless, even in this subgroup, median progression-free survival in the IRIS group was 6·0 months and no worse than that previously reported for second-line chemotherapy in patients refractory to fluorouracil alone.[3,24–26]

In each of the subgroups stratified by use or non-use of oxaliplatin, no differences were identified in other patient characteristics between the two groups. There is no clearly understood reason for the interaction between the presence or absence of oxaliplatin and therapeutic effects in the two groups. We speculate that a different mode of fluorouracil administration in FOLFIRI compared with prior therapy might work more effectively than S-1 for the patients without prior therapy with oxaliplatin, and that S-1 might have some salvage effects in patients who received FOLFOX involving bolus and infusional fluorouracil.

Our data have some limitations. First, progression-free survival, the primary endpoint, was assessed on the basis of disease progression established by the investigator at each medical institution. Therefore, caution should be used when our results are compared with those of other studies in which progression-free survival was centrally assessed. Second, around 40% of the patients in this trial were not previously treated with oxaliplatin, since FOLFOX therapy was approved in Japan only just before the study was started. Because FOLFOX is now widely used as first-line chemotherapy in Japan, patients should be carefully selected when our overall results are used to apply IRIS therapy in the clinical setting. However, we believe that the findings from subgroup analyses suggest that IRIS was better than FOLFIRI in patients who received an oxaliplatin-containing regimen as first-line chemotherapy.

In conclusion, progression-free survival with IRIS is not inferior to that with FOLFIRI in patients receiving second-line chemotherapy for metastatic colorectal cancer. IRIS therapy can be an additional treatment option for second-line chemotherapy in metastatic colorectal cancer.

Research in context

Systematic review

Before the study was initiated, we searched the PubMed database for relevant articles using search terms such as "metastatic colorectal cancer", "chemotherapy", "second line", and "phase 3". Based on the relevant articles obtained, the institutional review board reviewed the appropriateness as well as ethical and scientific aspects of the study, on which to base the approval of the study.

Interpretation

Our study demonstrates the non-inferiority of IRIS to FOLFIRI, one of international standard therapies for second-line chemotherapy of metastatic colorectal cancer; thus, IRIS is an option for second-line chemotherapy.

Contributors

IH, SM, NB, YS, HT, YK, MW, and KS, as a steering committee, participated in all phases of this study, including design and writing of the ancillary protocol, analysis, interpretation, and preparation of the report. All authors, with the exception of IH and SM, participated in data collection. SM undertook all analyses. All authors reviewed and helped revise the paper, and approved the submitted version. A list of participating institutions can be found in the webappendix.

Conflicts of interest

KM has received payment for writing the report from Daiichi Sankyo and honoraria from Taiho and Yakult Honsha. NB has received a grant from Taiho; NB's institution has received grants from Taiho. YS has received honoraria from Taiho and Yakult Honsha; YS's institution has received board membership fees and grants from Daiichi Sankyo. AT has received honoraria from Taiho, Daiichi Sankyo, and Yakult Honsha;

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AT's institution has received grants from Taiho, Daiichi Sankyo, and Yakult Honsha. SS has received honoraria from Yakult Honsha and lecture fees from Taiho; SS's institution has received grants from Taiho. HB has received board membership fees from Taiho and Daiichi Sankyo, and lecture fees from Taiho, Daiichi Sankyo, Yakult Honsha, and Wyeth; HB's institution has received grants from Taiho, Daiichi Sankyo, Yakult Honsha, Kyowa Hakko Kirin, and Wyeth. TS has received consulting fees from Taiho, honoraria and lecture fees from Taiho, Daiichi Sankyo, and Yakult Honsha, and lecture fees from Kyowa Hakko Kirin and Wyeth; TS's institution has received grants form Taiho. TD has received honoraria from Taiho, Wyeth, and Yakult Honsha, and lecture fees from Taiho, Daiichi Sankyo, Wyeth, and Yakult Honsha; TD's institution has received grants from Taiho and Yakult Honsha. KI's institution has received grants from Taiho. TN has received honoraria from Taiho, Wyeth, and Yakult Honsha; TN's institution has received grants from Daiichi Sankyo. KY has received lecture fees from Taiho, Daiichi Sankyo, Wyeth, and Yakult Honsha; KY's institution has received grants from Taiho. HT has received board membership fees from Daiichi Sankyo, consulting fees from Yakult Honsha, and honoraria from Taiho and Daiichi Sankyo. TE has received lecture fees from Kyowa Hakko Kirin, Taiho, Wyeth, and Yakult Honsha; TE's institution has received grants from Taiho and Yakult Honsha. ST's institution has received grants from Taiho. HK has received honoraria from Taiho, Daiichi Sankyo, and Yakult Honsha; HK's institution has received grants from Taiho and Daiichi Sankyo, HK's institution has received grants from Daiichi Sankyo. YK has received board membership fees from Daiichi Sankyo, Kyowa Hakko Kirin, Taiho, and Wyeth, and honoraria from Taiho, Daiichi Sankyo, and Yakult Honsha; YK's institution has received grants from Taiho, Daiichi Sankyo, and Yakult Honsha. MW has received board membership fees, honoraria, and lecture fees from Taiho; MW's institution has received grants from Taiho. IH has received board membership fees from Taiho, and consulting fees, honoraria, and lecture fees from Taiho, Daiichi Sankyo, and Yakult Honsha, and lecture fees from Kyowa Hakko Kirin. SM has received board membership fees from Daiichi Sankyo, consulting fees and honoraria from Taiho and Daiichi Sankyo; SM's institution has received grants from Daiichi Sankyo. KS has received board membership fees from Taiho, honoraria and lecture fees from Taiho, Daiichi Sankyo, and Yakult Honsha, and lecture fees from Wyeth; KS's institution has received grants from Taiho.

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interpreted in the context of other efficacy measures such as progression or disease-free survival, and, most importantly, experimental therapies are ultimately assessed in randomised comparative trials.

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S-1 for advanced colorectal cancer: do we need another oral fluorouracil prodrug?

See Articles page 853

Fluorouracil was one of the earliest developed cytotoxic drugs and is routinely used in the clinic for first-line, salvage, and adjuvant treatment of colorectal cancer. Oral fluorouracil prodrugs are of major practical advantage over infusional fluorouracil regimens,[1,2] which need ports and pumps, particularly for palliative treatment of advanced disease, for which patient-friendly treatment options are of the utmost importance to help patients to have the best quality of life in their remaining months. Despite these advantages, capecitabine is not used often enough, with the exception of specific regions—eg, Italy and the UK—in part because of reimbursement systems that often incentivise treatment by infusion rather than with tablets. Thus, the appearance of another oral fluorouracil prodrug should be appreciated, especially if this drug has proven similar efficacy to infusional fluorouracil, and does not add to the burden of toxic effects.

In this issue of *The Lancet Oncology*, Muro and colleagues[3] report the first randomised trial comparing the oral fluorouracil derivative S-1 with infusional fluorouracil for second-line treatment of advanced colorectal cancer after fluorouracil or FOLFOX failure. The study showed that S-1 plus irinotecan (IRIS) was non-inferior to the established FOLFIRI (fluorouracil, folinic acid, and irinotecan) regimen in terms of progression-free survival (PFS; the primary endpoint) and overall survival; thus, at least formally, S-1 has been proven to be an acceptable substitute for infusional fluorouracil in this second-line setting. However, the study population might not be indicative of the general population of patients needing second-line treatment for colorectal cancer. Because oxaliplatin was not licensed for use in Japan at the start of the study, only 60% of patients had oxaliplatin-containing regimens as first-line treatment, with some of the remaining 40% of patients receiving fluorouracil monotherapy. This issue could bias results, since first-line treatment with a combination regimen is a strong prognostic factor for impaired PFS under any second-line treatment.

Indeed, the preplanned but retrospective subgroup analysis stratifying by previous treatment with oxaliplatin is more confusing than supportive of the overall results. For patients who had not previously been exposed to oxaliplatin, IRIS seemed to be less effective than FOLFIRI (HR 1·490 for PFS; 1·302 for overall survival), whereas in the group with previous exposure to oxaliplatin, IRIS seemed to be more potent than FOLFIRI (HR 0·876 for PFS, 0·781 for overall survival). However, in view of the low numbers of patients in each of these subgroups, and therefore the lack of significance for these trends, one could question what these controversial results could mean, and if they are relevant enough to serve as a hypothesis for prospective investigation in another trial. The authors' speculation regarding the potential mechanism behind these results is interesting, however, they might have arisen by chance. There is a risk that, on the basis of these results, oncologists might falsely prefer S-1 instead of infusional

fluorouracil in patients who have not responded to first-line FOLFOX, despite the absence of statistical proof.

In view of the efficacy results presented by Muro and colleagues, the next question is whether S-1 has benefits in terms of side-effects over infusional fluorouracil or capecitabine. Muro and co-workers reported an increased risk of grade 3 diarrhoea with IRIS compared with FOLFIRI (20·5% *vs* 4·7%), which seems higher than the more recent capecitabine/irinotecan regimen, which used a similar dose of irinotecan.[4,5] Western and in particular American populations seem to experience more diarrhoea with oral fluorouracil derivatives than do Asian patients; thus there is potential for a high risk of clinically significant diarrhoea—of particular concern in a palliative setting—with IRIS in a western population. This toxic effect could render the IRIS regimen unsuitable for combination with epidermal-growth-factor-receptor inhibitors, although this side-effect is probably not prohibitive for combination with bevacizumab. The absence of grade 3–4 hand-foot syndrome in this trial is encouraging, however this observation does not outweigh the risk of grade 3 or worse diarrhoea in a fifth of patients.

In conclusion, the results of this trial might allow us to add S-1 to the treatment armamentarium for patients with advanced colorectal cancer, at least for Asian populations. However, many further investigations are needed to address questions regarding dosing, patient population, and drug combinations, as well as in other treatment lines.

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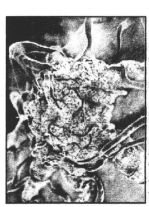
Is there really a yin and yang to VEGF-targeted therapies?

The discovery of vascular endothelial growth factor (VEGF) as a critical factor in tumour angiogenesis and growth has provided the foundation for the development of numerous treatments that inhibit the activity of this pathway. The fact that VEGF heterozygous knockout leads to embryonic lethality shows the importance of this pathway in vascular development and homoeostasis. VEGF-targeted treatments are now routinely used in a number of cancer types. However, initial expectations for anti-angiogenic treatments—eg, prolonged tumour dormancy, minimum toxic effects, and effectiveness in all tumour types—have not been met. More research is necessary to understand the role of VEGF in tumour biology and how VEGF biology synergises with other angiogenic mechanisms to induce and maintain the tumour vasculature.

Because of the critical nature of VEGF in homoeostasis, inhibition of its activity results in upregulation of other cytokines and growth factors.[1] This induction could be a direct consequence of cells sensing a loss of VEGF signalling, or could be secondary to the effects of VEGF inhibition in the tumour microenvironment, including induction of hypoxia, hypoxia-inducing factors, and downstream pathways. Preclinical studies have shown that VEGF-targeted treatments can increase tumour-cell migration, invasion, and metastasis.[2–4] Additionally, preclinical studies have shown that withdrawal of VEGF-targeted treatment can lead to a rapid re-growth of tumour vessels and tumour growth.[5] These preclinical studies have caused oncologists and patients to pause and consider the possibility that VEGF-targeted treatment might be detrimental to patients.

If the above is true, then we are caught in a "catch-22", whereby VEGF-targeted drugs can increase metastasis, but withdrawal of such treatment might result in explosive growth of tumours that might be "addicted" to VEGF inhibition. In phase 3 clinical trials, VEGF-targeted therapies have led to improved progression-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See Reflection and Reaction page 811

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Original Article

Phase I/II Study of Capecitabine Plus Oxaliplatin (XELOX) Plus Bevacizumab As First-line Therapy in Japanese Patients with Metastatic Colorectal Cancer

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Objective: The addition of bevacizumab to fluoropyrimidine-based combination chemotherapy as first-line therapy for metastatic colorectal cancer results in clinically significant improvements in patient outcome. However, clinical trials have been conducted primarily in Caucasian patients with only a small proportion of Asian patients. This Phase I/II study was designed to evaluate the efficacy and safety of XELOX (capecitabine plus oxaliplatin) plus bevacizumab in Japanese patients with metastatic colorectal cancer.

Methods: Patients with previously untreated, measurable metastatic colorectal cancer received bevacizumab 7.5 mg/kg and oxaliplatin 130 mg/m^2 on day 1, plus capecitabine 1000 mg/m^2 twice daily on days 1–14, every 3 weeks. A three-step design evaluated in: step 1, initial safety of XELOX in six patients; step 2, initial safety of XELOX plus bevacizumab in six patients; and step 3, efficacy and safety in a further 48 patients. The primary study endpoints were safety and response rate.

Results: No dose-limiting toxicity occurred during Steps 1 and 2. Fifty-eight patients were enrolled in Steps 2 and 3 and received XELOX plus bevacizumab. In the 57 patients assessed for response, the overall response rate was 72% (95% confidence interval, 58.5–83.0). Median progression-free survival was 11.0 months (95% confidence interval, 9.6–12.5) and median overall survival was 27.4 months (95% confidence interval, 22.0–not calculated). Eight patients (14%) underwent surgery with curative intent. The most common grade 3/4 adverse events were neurosensory toxicity (17%) and neutropenia (16%).

Conclusions: XELOX plus bevacizumab is effective and has a manageable tolerability profile when given to Japanese patients with metastatic colorectal cancer.

Key words: xelox – bevacizumab – colorectal cancer – Japanese

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