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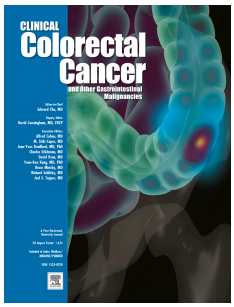

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Prognostic impact of primary tumor location on clinical outcomes of metastatic colorectal cancer treated with cetuximab plus oxaliplatin-based chemotherapy: a subgroup analysis of the JACCRO CC-05/06 trials

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Author Y.S. has received honoraria from Taiho Pharmaceutical, Chugai Pharma, Yakult Honsha, Takeda, and Merck Serono. Author W.I. has received consulting fees from Merck Serono, Daiichi Sankyo, Zeria Pharmaceutical, and Ono Pharmaceutical; research funding from Merck Serono, Taiho Pharmaceutical, Takeda, and Eisai; and honoraria from Taiho Pharmaceutical, Merck Serono, Chugai Pharma, Daiichi Sankyo, Takeda, Nippon Kayaku and Sawai Pharmaceutical Co. Author A.T. has received honoraria from Daiichi Sankyo, Taiho Pharmaceutical, Chugai Pharma, and Merck Serono. Author M.N. has received honoraria from Merck Serono, Takeda, Chugai Pharma, Taiho Pharmaceutical, Nippon Kayaku, Novartis, Yakult Honsha, Lilly Japan, Bristol-Myers Squibb, Bayer, Ajinomoto, Shionogi, Pfizer, and Ono Pharmaceutical. Author M.K. has received honoraria from Merck Serono, Takeda, Chugai Pharma, and Yakult Honsha. Author M.T. has received consulting fees from Taiho Pharmaceutical, Shionogi, AbbVie GK, Astra Zeneca Co., and Hisamitsu Pharma Co.; and honoraria from Mitsubishi Tanabe Pharma. The other authors have declared no conflicts of interest.

Micro-Abstract

Primary tumor location is a prognostic factor in metastatic colorectal cancer (mCRC). We assessed the prognostic impact of tumor location on survival and the association between *BRAF* mutation and tumor sidedness in mCRC patients treated with cetuximab. Our study showed that tumor location is a prognostic marker for first-line cetuximab plus oxaliplatin-based chemotherapy, irrespective of *BRAF* status.

Abstract

Purpose: Primary tumor location is a critical prognostic factor in metastatic colorectal cancer (mCRC); however, it remains unclear whether tumor location is a predictor of the response to cetuximab treatment. It is also uncertain if *BRAF* mutation contributes to the impact of tumor location on survival. We assessed the prognostic impact of tumor location on clinical outcomes in mCRC patients treated with first-line cetuximab chemotherapy.

Patients and Methods: The associations of tumor location with overall survival (OS) and progression-free survival (PFS) were evaluated in mCRC patients with *KRAS* exon 2 wild-type tumors who were enrolled in 2 clinical trials: JACCRO CC-05 of cetuximab plus FOLFOX (n=57, UMIN000004197) and CC-06 of cetuximab plus SOX (n=61, UMIN000007022). Tumors proximal or from splenic flexure to rectum were defined as right-sided or left-sided, respectively. In addition, exploratory RAS and *BRAF* mutation analyses were performed.

Results: A total of 110 patients were assessable for tumor location: 90 had left-sided tumors. Left-sided tumors were significantly associated with longer overall survival (36.2 vs. 12.6 months, HR 0.28, $P<0.0001$) and progression-free survival (11.1 vs. 5.6 months, HR 0.47, $P=0.0041$) than right-sided tumors; similar results were obtained in multivariate analysis. A sub-analysis showed that the association was evident in the FOLFOX group and that tumor location was an independent prognostic factor irrespective of *BRAF* status in RAS wild-type patients.

Conclusion: Our results suggest that primary tumor location might be a predictor of survival independent of *BRAF* status in mCRC patients who receive first-line cetuximab combined with oxaliplatin-based chemotherapy.

Key words: colorectal cancer, cetuximab, prognostic marker, tumor location

Introduction

Several factors have been identified as prognostic biomarkers in colorectal cancer (CRC), including germline mutations in DNA mismatch repair genes in stage II/III disease and *BRAF*^{V600E} mutations in stage IV disease.[1, 2] Moreover, mortality has been shown to be higher in proximal than in distal colon cancer.[3-7] The proximal, right-sided colon originates from the embryonic midgut and is served by the superior mesenteric artery, whereas the distal, left-sided colon arises from the hindgut and is perfused by the inferior mesenteric artery.[8] There are differences in epidemiologic, clinical, and histological features between right-sided and left-sided CRCs.[3] Such differences probably lead to the disparity in outcomes according to the primary tumor site.

Right-sided and left-sided CRCs differ with respect to their molecular pathways of carcinogenesis, biological characteristics, and genomic patterns. Right-sided tumors express the CIMP phenotype and are more likely to be characterized by MSI-high, CpG island methylation, and *BRAF* mutations.[9-12] In contrast, left-sided tumors have a phenotype that involves chromosomal instability and are characterized by loss of heterozygosity and *TP53* mutations.[9] Several microarray studies have found over 1000 genes that show different expression patterns between right- and left-sided tumors, potentially leading to distinct embryonic origins and postnatal regulation.[13-15]

Sub-analyses of randomized clinical trials performed in the United States and Europe have demonstrated that primary tumor location is a critical prognostic factor in metastatic CRC (mCRC) treated by first-line chemotherapy combined with biological agents.[16-19] In the FIRE-3 trial, which compared FOLFIRI plus cetuximab with FOLFIRI plus bevacizumab as first-line therapy in mCRC patients with *KRAS* exon 2 wild-type tumors, a sub-analysis

indicated that left-sided tumors were associated with significantly longer overall survival (OS) and progression-free survival (PFS) than right-sided tumors among patients with RAS wild-type tumors who received cetuximab.[18] The impact of tumor sidedness on clinical outcomes in cetuximab-treated patients was also demonstrated in a sub-analysis of the CALGB/SWOG 80405 trial; however, other RAS mutations were not taken into account and potential effects of treatment-related differences between the oxaliplatin-treated patients and irinotecan-treated patients could not be excluded.[19] In addition, it remains unclear if *BRAF* mutations contribute to the impact of tumor location on survival. To our knowledge, the impact of primary tumor location on outcomes has not been assessed in Asian patients enrolled in prospective clinical trials.

We therefore investigated the prognostic impact of primary tumor location on the clinical outcomes of Japanese patients with *KRAS* exon 2 wild-type mCRC who were enrolled in the JACCRO CC-05 (UMIN000004197) or CC-06 (UMIN000007022) trial, which evaluated the efficacy of cetuximab combined with either FOLFOX or SOX as first-line treatment. Moreover, we attempted to assess the effect according to RAS and *BRAF* status in preparation for forthcoming molecular analyses.

Patients and Methods

Patient population

We studied mCRC patients who were enrolled in the phase II JACCRO CC-05 or CC-06 trial, evaluating first-line cetuximab treatment combined with FOLFOX or SOX, respectively, and were assessable for primary tumor location (**Figure 1**). The identical eligibility criteria of the 2 trials was as follows: adenocarcinoma of the colon or rectum with

immunohistological expression of EGFR; *KRAS* exon 2 wild-type tumor with unresectable metastases; at least one measurable lesion of 10 mm or a residual nonmeasurable lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1; adequate bone marrow function (hemoglobin >9.0 g/dl, neutrophil count >1,500/mm^3, platelet count >100,000/mm^3), hepatic function, and renal function; an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1; and an age of 20 to 79 years. Patients with uncontrolled infection, massive ascites or pleural effusion, symptomatic brain metastases or other malignancies within 5 years before enrollment (with the exception of early carcinoma that had been treated with curative intent), a history of systemic chemotherapy for mCRC, or previous treatment with oxaliplatin or cetuximab were excluded. The studies were conducted in accordance with the Declaration of Helsinki and were approved by the ethical committee of each participating institute. Written informed consent was obtained from all patients before enrollment.

Tumors located from the cecum to the hepatic flexure were classified as right-sided, while tumors that included the splenic flexure, descending colon, sigmoid colon, and rectum were classified as left-sided. Patients with tumors in the transverse colon or synchronous right-sided and left-sided tumors were excluded from analysis.

Chemotherapy

In the FOLFOX-regimen (JACCRO CC-05), on day 1 of each 14-day treatment cycle, patients received cetuximab (a 120-min infusion of 400 mg/m^2 of body surface area [BSA] followed by a 60-min infusion of 250 mg/m^2 of BSA given weekly thereafter) plus modified-FOLFOX6 (a 120-min infusion of oxaliplatin at a dose of 85 mg/m^2 of BSA; a 120 min

infusion of leucovorin [LV] at a dose of 200 mg/m² of BSA; and an intravenous bolus of fluorouracil [FU] at a dose of 400 mg/m² of BSA followed by a continuous 46-h infusion of 2400 mg/m² of BSA). In the SOX-regimen (JACCRO CC-06), on day 1 of each 21-day treatment cycle, patients received cetuximab (a 120-min infusion of 400 mg/m² of BSA followed by a 60-min infusion of 250 mg/m² of BSA given weekly thereafter) plus SOX (a 120-min infusion of oxaliplatin at a dose of 130 mg/m² of BSA on day 1; and oral S-1 at a dose of 80 mg/m² of BSA on days 1-15). Pretreatment with dexamethasone and a histamine-1 blocker was strongly recommended to prevent allergic or hypersensitivity reactions to cetuximab. Treatment was continued until disease progression, unacceptable toxic effects developed, a complete response was achieved, surgical resection became possible, or the patient requested or the physician decided that therapy should be withdrawn. Dose modifications of chemotherapy and cetuximab were permitted according to protocol-defined criteria. In the event of grade 3 or 4 allergic or hypersensitivity reaction, cetuximab or oxaliplatin was permanently discontinued.

Assessment of efficacy

The primary endpoint of these 2 phase II trials was the proportion of patients who had an objective response (complete or partial response). Secondary endpoints included early tumor shrinkage (ETS) (percentage change in the size of target lesions as compared with the baseline value), evaluated every 8 weeks until progression, PFS based on disease progression detected by external review or death from any cause, OS, secondary resection of metastases with curative intent, and safety. Responses were evaluated according to RECIST, version 1.1 by the investigators and were then validated by an external review board. ETS

was defined as a minimal tumor reduction of 20% at 8 weeks.

DNA isolation and RAS/BRAF mutation analysis

Formalin-fixed, paraffin-embedded (FFPE) tumor specimens were cut into sections with a thickness of 3 or 10 μm. In a preparation for macrodissection, one 3-μm slide was stained with H&E and was then evaluated for tumor content and marked for areas with dominant tumor foci by a pathologist. Macrodissection was performed by scratching the marked areas with a blade to ensure that as many tumor cells as possible were dissected. The dissected particles of tissue were transferred to reaction tubes for isolation of genomic DNA. Genomic DNA was extracted from the FFPE tissue derived from the tumor samples with the use of a QIAamp DNA FFPE Tissue Kit (QIAGEN KK) according to the manufacturer's protocol.

RASKET KIT (MBL, Nagoya, Japan) was used in accordance with the manufacturer's protocol to detect *KRAS*/*NRAS* exon 2 mutations (G12S, G12C, G12R, G12D, G12V, G12A, G13S, G13C, G13R, G13D, G13V, and G13A), eight types of *KRAS*/*NRAS* exon 3 mutations (A59T, A59G, Q61K, Q61E, Q61L, Q61P, Q61R, and Q61H), and four types of *KRAS*/*NRAS* exon 4 mutations (K117N, A146T, A146P, and A146V). *BRAF* V600E mutations were detected by dye terminator sequencing. Exon 15 of the *BRAF* gene was amplified by polymerase chain reaction (PCR), and the PCR products were then visualized using agarose gel electrophoresis with ethidium bromide staining. The products were directly sequenced with the use of an ABI 3130xl Genetic Analyzer (Thermo Fisher Scientific K.K., Yokohama, Japan) according to the manufacturer's instructions.

This study was conducted in accordance with the REporting recommendations for

tumor MARKer prognostic studies (REMARK).[20, 21] Tissue analyses were performed at SRL, Inc. (Tokyo, Japan) and G&G Science Co., Ltd. (Fukushima, Japan) after obtaining approval from the Institutional Review Board of each institution that participated in the JACCRO CC-05/06AR trials (UMIN000010635).

Statistical evaluation

The prognostic impact of primary tumor location on outcomes was investigated in all subgroups of enrolled patients in addition to the cohort of patients with RAS wild-type tumors. The primary endpoint of the present study was OS. Objective response rate (ORR), ETS (yes, no), and PFS were the secondary endpoints. The associations of primary tumor location with tumor response or ETS were examined using Fisher's exact test. The associations between tumor location and OS or PFS were assessed using Kaplan-Meir curves and log-rank tests in univariate analyses. A multivariable Cox regression model was used to evaluate the independent effects of a marker on OS or PFS and to adjust for ECOG PS (0 vs. 1), the number of organs involved (1 vs. ≥2), and primary tumor site (right vs. left), all of which were significantly associated with outcomes (P<0.05).

SAS 9.0.3 software (SAS Institute, Cary, NC, USA) was used to perform all analyses. All tests were 2-sided with a significance level of 0.05.

Results

Patient characteristics

A total of 110 patients were enrolled. Ninety (82%) of the patients had left-sided tumors, and 20 (18%) had right-sided tumors. The patient characteristics are summarized in **Table 1**.

Primary tumor was resected among 75% of patients in each side of tumor location. There were no statistically significant differences in characteristics between patients with left-sided tumors and those with right-sided tumors (**Supplementary Table 1**). In the enrolled patients, the ORR was 65% (95% CI 57%–74%). The median PFS and OS were 9.4 months (95% CI 8.1–11.3 months) and 33.9 months (95% CI 26.5–43.6 months), respectively.

Clinical outcomes according to primary tumor location

The ORR was 69% (95% CI 59%-78%) in patients with left-sided tumors and 50% (95% CI 28%-72%) in those with right-sided tumors. There was no significant difference in the ORR between the left-tumor cohort and right-tumor cohort (*P*=0.12). ETS also did not differ significantly between the 2 cohorts (78% vs. 74%, *P*=0.76). The median PFS and OS were significantly longer in the left-sided tumor cohort than the right-sided tumor cohort (11.1 months vs. 5.6 months, HR 0.47, *P*=0.0041; 36.2 months vs. 12.6 months, HR 0.28, *P*<0.0001, respectively) (**Table 2, Figure 2**).

Multivariate analysis adjusted for ECOG PS, the number of organs involved, and tumor location revealed that primary tumor location was an independent prognostic factor for both PFS and OS (HR 0.48, *P*=0.0041; HR 0.31, *P*<0.0001, respectively) (**Table 3**).

Subgroup analysis according to backbone chemotherapeutic regimen and RAS/BRAF status

We performed a subgroup analysis stratified according to regimen (modified-FOLFOX6 or SOX). An association of tumor location with clinical outcomes was evident in the FOLFOX group (*P*=0.0002 for PFS and *P*<0.0001 for OS), but was not significant in the SOX group

(*P*=0.30 for PFS and *P*=0.079 for OS). In patients with right-sided tumors, median PFS and OS were numerically longer in the SOX group than in the FOLFOX group (6.7 months vs. 3.0 months, 18.3 months vs. 5.7 months, respectively) (**Supplementary Figure 1**).

Among the 110 patients, external analysis of RAS mutations was conducted in 71 patients from whom tumor samples were available for RAS and *BRAF* molecular analyses. RAS and *BRAF* mutations were successfully analyzed in 67 (94%) of the 71 patients. The analyses detected RAS mutations in 7 (10%) of the 67 patients and *BRAF* mutations in 9 (15%) patients. In 60 patients with RAS wild-type tumors, left-sided tumors were significantly associated with longer PFS and OS than right-sided tumors (11.3 months vs. 5.6 months, HR 0.49, *P*=0.061; 42.8 months vs. 18.3 months, HR 0.21, *P*=0.0001, respectively). Moreover, in patients with RAS/*BRAF* wild-type tumors, left-sided tumors were associated with significantly longer OS than right-sided tumors (*P*=0.039) (**Table 4**). Left-sided tumors were significantly associated with OS on multivariate analysis adjusted for factors including *BRAF* status in patients with RAS wild-type tumors (HR 0.21, *P*=0.0018), suggesting that tumor location can serve as a prognostic marker irrespective of *BRAF* status.

Discussion

Our study demonstrated that primary tumor location is a predictor of survival in mCRC patients who receive first-line cetuximab plus oxaliplatin-based chemotherapy. Our results potentially confirm the prognostic impact of tumor location in mCRC. Moreover, our findings suggest that primary tumor location is an independent prognostic biomarker irrespective of *BRAF* status.

Several studies have revealed that left-sided tumors are present more often than

right-sided tumors in early-stage disease.[4, 22-25] A sub-analysis of the database of the Surveillance, Epidemiology, and End Results (SEER) program, a large epidemiological study, reported that right-sided tumors are associated with poorer outcomes than left-sided tumors after adjustment for tumor stage,[4, 5] indicating that primary tumor location can potentially be used as a prognostic marker in CRC. A sub-group analysis of the AIO KRK-0104 trial has shown favorable survival of mCRC patients with left-sided tumors who received first-line combination chemotherapy with cetuximab.[26] In addition, multivariate Cox regression analyses of PFS and OS in the FIRE-3 trial demonstrated that left-sided tumor location is a predictor of favorable outcomes in RAS wild-type mCRC patients who receive first-line FOLFIRI plus cetuximab.[18] However, there has been no report regarding the impact of tumor sidedness on survival in patients who receive only oxaliplatin-based chemotherapy as a platform regimen plus cetuximab. Our results support the findings of previous studies and further indicate that primary tumor location can serve as a predictor of survival in mCRC patients treated with first-line cetuximab combined with oxaliplatin-based chemotherapy. In our study, the primary tumor location was unrelated to tumor response as an outcome of treatment. A sub-analysis of the FIRE-3 trial indicated a significant effect of primary tumor location on ORR (OR 2.7, P=0.019).[18] However, in the AIO KRK-0104 study, left-sided tumors were not significantly associated with a higher ORR as compared with right-sided tumors (64% vs. 43%, P=0.12) in patients with *KRAS* exon 2 wild-type tumors.[26] The relation between tumor location and ORR thus remains controversial.

BRAF mutation is a strong negative prognostic determinant in mCRC, and patients with *BRAF*-mutated tumors have an extremely poor life expectancy.[27, 28] The frequency of *BRAF* mutations in CRC significantly increases linearly along the bowel from the rectum to

ascending colon, rather than changing abruptly at the splenic flexure.[12] Right-sided tumors are therefore more likely to be characterized by *BRAF* mutations. The impact of sidedness on survival may reflect the contribution of *BRAF* mutation to poor outcomes; however, a sub-analysis of a clinical trial showed that tumor sidedness has a prognostic effect on outcomes regardless of *BRAF* status.[16] We analyzed the association between tumor location and clinical outcomes according to RAS and *BRAF* status. In patients with RAS wild-type as well as those with RAS/*BRAF* wild-type tumors, left-sided tumors were significantly associated with longer OS. Moreover, a multivariate analysis adjusted for factors including *BRAF* status revealed that tumor location had a prognostic effect on survival in patients with RAS wild-type tumors, suggesting that primary tumor location is an independent prognostic biomarker irrespective of *BRAF* status. This finding needs to be confirmed in larger studies.

Our study group comprised patients who received FOLFOX or SOX as backbone chemotherapy. We therefore additionally analyzed the association of tumor location with clinical outcomes according to regimen (FOLFOX or SOX). The association was significant in the FOLFOX group (P=0.0002 for PFS and P<0.0001 for OS), but was not significant in the SOX group (P=0.30 for PFS and P=0.079 for OS). Interestingly, median PFS was numerically longer in the SOX group than in the FOLFOX group in patients with right-sided tumors (6.7 months vs. 3.0 months). In contrast, in patients with left-sided tumors median PFS was numerically shorter in the SOX group than in the FOLFOX group (9.2 months vs. 11.3 months). In the SOFT trial, which showed that SOX plus bevacizumab was noninferior to FOLFOX plus bevacizumab as first-line treatment for mCRC, the subgroup analysis of PFS indicated no significant interaction between the regimen and primary tumor location (colon vs. rectosigmoid vs. rectum).[29] Additional analyses according to tumor sidedness (left vs. right)

may provide informative evidence on the relations between the effectiveness of S-1 and tumor location in trials evaluating S-1-based regimens.

It remains controversial whether left-sidedness of primary tumors is a predictor of the response to cetuximab in patients with *KRAS*/RAS wild-type mCRC. A recent study that reanalyzed data from the NCIC CO.17 trial has reported that tumor location is a strong predictor of a beneficial effect of cetuximab therapy on PFS. Among patients with *KRAS* exon 2 wild-type colon cancer, cetuximab treatment was associated with significantly better PFS than best supportive care only in patients with left-sided tumors (median 5.4 months vs. 1.8 months, HR 0.28, *P*<0.0001) and not in patients with right-sided tumors (median 1.9 months vs. 1.9 months, HR 0.73, *P*=0.26) [interaction *P*=0.002].[30] Retrospective analyses of the FIRE-3 and CALGB/SWOG80405 trials showed that treatment effects in patients with left-sided tumors were more prominent in the cetuximab arm: chemotherapy plus cetuximab significantly improved OS as compared with chemotherapy plus bevacizumab in patients with KRAS/RAS wild-type mCRC who had left-sided tumors, whereas the benefit of treatment with cetuximab appeared to be limited in patients who had right-sided tumors.[18, 19] In our study, multivariate analysis showed that tumor location was significantly associated with PFS. Further studies are needed to assess the predictive relevance of primary tumor location in patients with RAS wild-type mCRC treated with first-line cetuximab-containing regimens.

Although the number of patients included in this pooled analysis of 2 prospective trials was relatively small, our results are consistent with the findings of previous studies. However, because RAS and *BRAF* tests were performed using samples that were retrospectively collected from patients for whom tumor-tissue specimens were available, a selection bias cannot be excluded. Therefore, the prognostic impact of primary tumor location on clinical

outcomes should be confirmed in future studies of larger groups of patients.

Conclusion

Primary tumor location was significantly associated with survival in mCRC patients who received cetuximab plus oxaliplatin-based chemotherapy as first-line treatment. Patients with left-sided tumors had significantly better OS than those with right-sided tumors. Our results further indicate that the primary tumor location may be a viable prognostic marker regardless of *BRAF* status.

Clinical Practice Points

- Primary tumor location is a prognostic factor in metastatic colorectal cancer (mCRC); however, it remains unclear whether location of the primary tumor is a predictive marker of the response to cetuximab treatment.
- It is also unclear whether *BRAF* mutation contributes to the impact of tumor location on survival.
- In the present study, the prognostic impact of tumor location on survival and the association between *BRAF* mutation and tumor sidedness were investigated in mCRC patients who were enrolled in clinical trials and received first-line cetuximab combined with oxaliplatin-based chemotherapy.
- Left-sided tumors were associated with significantly longer OS and PFS than right-sided tumors; moreover, this association remained statistically significant in multivariate analysis.

- Sub-analyses showed that the association between tumor sidedness and outcomes was evident in the FOLFOX group rather than in the SOX group and that tumor location was an independent prognostic factor irrespective of *BRAF* status in patients with RAS wild-type tumors.
- Our study confirms the prognostic impact of tumor location on survival in mCRC patients treated with first-line cetuximab plus oxaliplatin-based chemotherapy. Moreover, our results suggest that tumor location might be a prognostic marker irrespective of *BRAF* status.

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Figure titles and legends

Figure 1. Flowchart of patient allocation to treatment groups.

Figure 2. Kaplan–Meier curves of clinical outcomes according to primary tumor location: A) progression-free survival, B) overall survival.

Supplementary Figure 1. Kaplan–Meier curves of clinical outcomes according to primary tumor location: A) progression-free survival, B) overall survival in the FOLFOX plus cetuximab group; C) progression-free survival, D) overall survival in the SOX plus cetuximab group.

Table 1. Patients' characteristics

Characteristic	All (*N*=110) *N*	%
Gender		
Male	63	57
Female	47	43
Age (years)		
Median (range)	61.5 (34-83)	
< 65	66	60
≥ 65	44	40
Performance Status		
ECOG 0	98	89
ECOG 1	12	11
Diagnosis		
Metachronous	26	24
Synchronous	84	76
Primary lesion		
Yes	27	25
No	83	75
Number of metastatic sites		
1	49	45
≥ 2	61	55
Previous adjuvant chemotherapy		
Yes	8	7
No	102	93
Regimen		
FOLFOX	52	47
SOX	58	53

Abbreviations: ECOG, Eastern Cooperative Oncology Group.

Table 2. Clinical outcomes according to primary tumor location

		Tumor response		Early tumor shrinkage		Progression-free survival		Overall survival	
	N	CR, PR	SD, PD	Yes	No	Median (95%CI) months	HR (95%CI)	Median (95%CI) months	HR (95%CI)
Right-sided	20	10 (50%)	9	14 (74%)	5 (26%)	5.6 (1.9-7.3)	1	12.6 (4.9-23.5)	1
Left-sided	90	62 (69%)	23	67 (78%)	19 (22%)	11.1 (9.1-13.5)	0.47 (0.28-0.80)	36.2 (27.5-45.3)	0.28 (0.15-0.52)
P			0.12		0.76		0.0041		<0.0001

* *P* values were based on Fisher's exact test for tumor response and early tumor shrinkage and log-rank test for PFS and OS in the univariate analysis.
Bold characters: significant

Table 3. Prognostic factors in multivariate analysis

	Progression-free survival			Overall survival		
	HR	95%CI	*P*-value*	HR	95%CI	*P*-value*
Gender						
Male vs. Female	1.22	0.78-1.89	0.76	1.14	0.66-1.97	0.96
ECOG PS						
0 vs. 1	0.69	0.37-1.30	0.20	0.50	0.23-1.07	0.12
Number of organs involved						
1 vs. ≥2	0.97	0.63-1.49	0.98	0.41	0.22-0.76	0.0025
Tumor location						
Left vs. Right	0.48	0.28-0.81	0.0041	0.31	0.17-0.58	<0.0001

* *P* values were based on Wald test for PFS and OS in the multivariable Cox regression model adjusted for ECOG PS, number of organs involved, and tumor location.

Table 4. Primary tumor location and clinical outcomes according to RAS/*BRAF* status

	KRAS exon 2 wt (n=110)		RAS wt (n=60)		RAS/*BRAF* wt (n=51)	
Location	Left	Right	Left	Right	Left	Right
Number	90	20	51	9	47	4
ORR (%)	69	50	78	56	79	50
	P=0.12		*P*=0.21		*P*=0.23	
Median PFS (m)	11.1	5.6	11.3	5.6	11.6	6.1
	HR 0.47 (0.28-0.80)		HR 0.49 (0.24-1.13)		HR 1.02 (0.36-4.28)	
	P=0.0041		*P*=0.061		*P*=0.97	
Median OS (m)	36.2	12.6	42.8	18.3	NR	18.3
	HR 0.28 (0.15-0.52)		HR 0.21 (0.09-0.53)		HR 0.29 (0.09-1.27)	
	P<0.0001		*P*=0.0001		*P*=0.039	

P values were based on Fisher's exact test for tumor response and early tumor shrinkage and log-rank test for PFS and OS in the univariate analysis.

Abbreviations: wt, wild-type; ORR, objective response rate; PFS, progression-free survival; OS, overall survival; NR, not reached

Figure 1

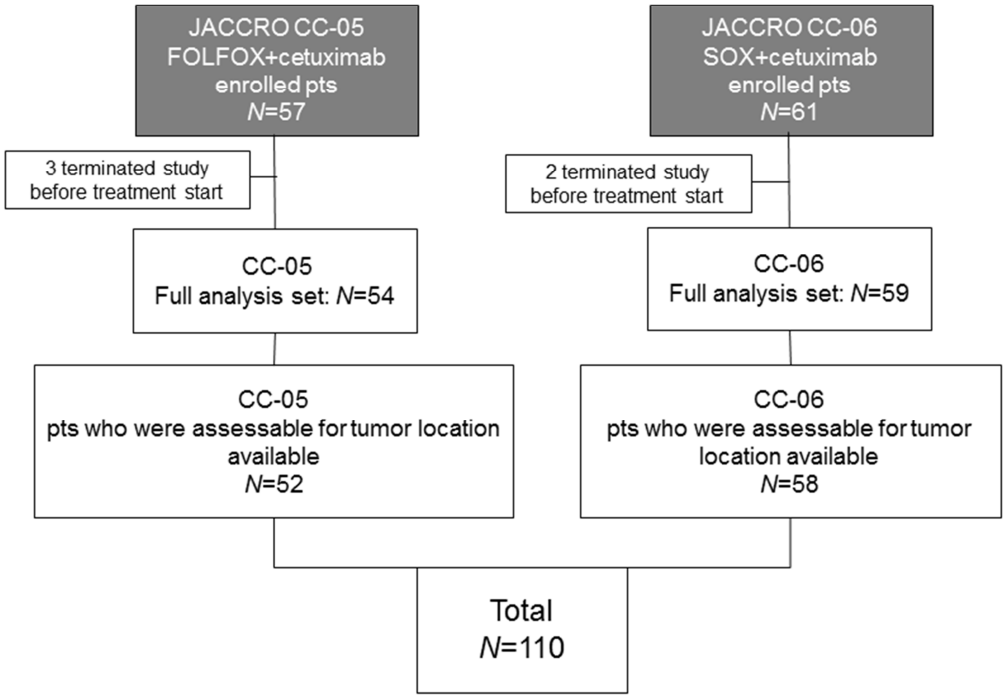
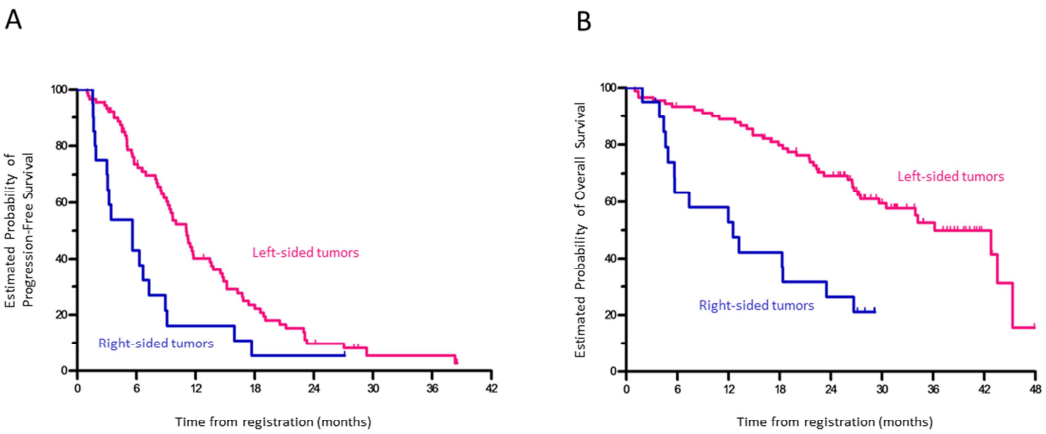


Figure 2



Supplementary Table 1. Patients' characteristics according to primary tumor location

	Left (*N*=90)	Right (*N*=20)	
	N (%)	*N* (%)	*P*-value
Gender			
Male	54 (60)	9 (45)	0.32
Female	36 (40)	11 (55)	
Age (year)			
Median (range)	61.5	61.5	0.55
< 65	53 (59)	13 (65)	0.80
≥ 65	37 (41)	7 (35)	
Performance Status			
ECOG 0	81 (90)	17 (85)	0.45
ECOG 1	9 (10)	3 15)	
Diagnosis			
Metachronous	22 (24)	4 (20)	0.78
Synchronous	68 (76)	16 (80)	
Primary lesion			
Yes	22 (24)	5 (25)	1.00
No	68 (76)	15 (75)	
Number of metastatic sites			
1	43 (48)	6 (30)	0.21
≥ 2	47 (52)	14 (70)	
Previous adjuvant chemotherapy			
Yes	7 (8)	1 (5)	1.00
No	83 (92)	19 (95)	
Regimen			
FOLFOX	43 (48)	9 (45)	1.00
SOX	47 (52)	11 (55)	

* Differences in patients' baseline characteristics between locations were examined using Fisher's exact test

Abbreviations; ECOG, Eastern Cooperative Oncology Group.

Supplementary Figure 1