Cetuximab Might Be Detrimental to Metastatic Colorectal Cancer Patients with *KRAS* Codon 12 Mutations

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Abstract. *Background: Anti-epidermal growth factor receptor (EGFR) monoclonal antibodies benefit patients with wild-type KRAS exon 2 metastatic colorectal cancer (mCRC). However, their effect in KRAS-mutant mCRC remains unclear. Patients and Methods: This was a retrospective study enrolling 163 patients with unresectable KRAS-mutant mCRC diagnosed at the National Taiwan University Hospital between 2007 and 2011. Results: The median overall survival (mOS) was 29.5 months in patients who had never used cetuximab and 19.0 months in those who had (p=0.040). The mOS was 32.0 months in patients with mutant KRAS codon 12 who had never used cetuximab and 17.5 months in those who had (p=0.017). In patients with mutant KRAS codon 13, the mOS was not significantly different. Univariate and multivariate Cox proportional hazards analysis revealed that absence of cetuximab treatment was an independent prognostic factor for longer mOS in patients with unresectable KRAS-mutant mCRC. Conclusion: Cetuximab usage might be detrimental to patients with mCRC with mutant KRAS codon 12.*

The clinical benefit of anti-epidermal growth factor receptor (EGFR) monoclonal antibodies (mAbs), such as cetuximab or panitumumab, in metastatic colorectal cancer (mCRC) with wild-type *KRAS* exon 2 tumors is well known (1-4). The CRYSTAL study was designed for patients with mCRC receiving irinotecan/fluorouracil/leucovorin (FOLFIRI) with/without mAbs to EGFR. Patients with tumors harboring a KRAS mutation who received FOLFIRI with cetuximab had a shorter median progression-free survival (PFS) period than those who received FOLFIRI alone (7.4 months *versus* 7.7 months, respectively) (2, 5). Similarly, in the PRIME trial, a randomized phase III study analyzing patients with *KRAS*-mutant mCRC treated with oxaliplatin/fluorouracil/leucovorin (FOLFOX) and with/without panitumumab, the median PFS in those receiving panitumumab was 7.3 months *versus* 8.8 months in patients receiving FOLFOX alone (6). Consistent with these studies, evidence of the deleterious effects of mAbs against EGFR has been mounting in additional prospective randomized trials (1-3, 5-11).

However, it seems that not all *KRAS*-mutant mCRC tumors display the same response to mAbs to EGFR. De Roock *et al.* demonstrated that patients with the G13D *KRAS*-mutant mCRC receiving cetuximab with/without chemotherapy had similar PFS as those whose tumors expressed wild-type *KRAS* (4 months *versus* 4.2 months, respectively) (12). In addition, patients with the G13D *KRAS*-mutant tumors that received cetuximab had a longer PFS than those with a codon 12 mutation (12). Furthermore, Andreyev *et al.* reported that patients with the G12V *KRAS*-mutant mCRC had significantly shorter overall survival (OS) than patients with other *KRAS* mutation subtypes (13). This heterogeneity in response to mAbs to EGFR for different KRAS-mutant subtypes was also observed in other studies (14-20).

Although most clinical guidelines suggest using mAbs to EGFR only in patients with mCRC expressing wild-type

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KRAS, the clinical data in support of these guidelines primarily derived from patients who received mAbs to EGFR as first-line treatment (2, 3, 6, 9, 21-24). Thus, we retrospectively collected the clinical data of patients with *KRAS*-mutant mCRC. Our primary study goal was to compare the OS for mCRC patients with KRAS mutant tumors who received mAbs to EGFR with those who did not. We further analyzed the response of various *KRAS* mutation subtypes to mAbs to EGFR and employed Cox regression analysis to identify independent factors that influence OS.

Patients and Methods

Patient enrollment. Between 2007 and 2011, the clinical data of patients who were diagnosed with all stages of CRC were retrieved from the Medical Information Management Office and the Cancer Registry Office of National Taiwan University Hospital (NTUH). Study subjects were further identified using the following inclusion criteria: diagnosis of unresectable mCRC; treatment of at least two cycles after enrollment; age of ≥18 years; tumors with known *KRAS* mutation subtype or a tissue sample for *KRAS* mutation subtypes analysis if the *KRAS* mutation subtype was unknown; and complete medical records, including regular computed tomograpic (CT) scan follow-up reports at NTUH. Patients were excluded if they had two or more active primary malignancies; they had tumors expressing wild-type *KRAS*; their *KRAS* mutation subtype was uncertain and could not be re-confirmed; they had received cetuximab-based treatment prior to enrollment; they had curable diseases or had been treated for curative intent; they had human immunodeficiency virus infection; or their medical records were incomplete. All patients were treated at the NTUH. This study was approved by the Institutional Review Board of NTUH (protocol #201104091RC), and informed consent was obtained from each participant.

Data collection. The following clinical data were collected from the detailed medical records: age at enrollment, gender, pathology reports and KRAS subtypes, date of initial CRC diagnosis at any stage, date of the enrollment, location of the primary CRC at initial diagnosis, treatment after enrollment, date of the death or the last follow-up at December 31, 2013. Because panitumumab is not currently available in Taiwan, the clinical data was stratified into two groups containing patients with and without a history of cetuximab treatment.

KRAS mutation test. The KRAS mutation status of the mCRC tumors was determined by direct sequencing at the NTUH Second Core Laboratory as previously described (25). Briefly, the DNA was extracted from paraffin-embedded specimens (QIAamp DNA Micro Kit Qiagen, Valencia, CA, USA). The primers for codons 12 and 13 of *KRAS* were then used. After polymerase chain reaction (PCR) amplification using an ABI PRISM 2700 or ABI 9700 PCR machine (Applied Biosystems, Foster City, CA, USA), the amplicons were purified using a gel/PCR DNA extraction kit (Geneaid, New Taipei City, Taiwan, ROC). The purified DNA was then cycle-sequenced using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Tumors whose *KRAS* mutation status was analyzed in other medical centers was also confirmed again at our center. If a discrepancy was detected, the result obtained by the NTUH was used in the final analysis.

Diagnosis and treatment. All diagnoses of CRC were made by two independent pathologists who reviewed all pathological specimens. Staging examinations included routine CT scans of the chest, abdomen, and pelvis. Other imaging studies, such as bone scans and positron emission tomography (PET), were ordered, if necessary. All systemic treatments were determined by the patients' physicians according to individual performance status and comorbidities. Tumors were routinely assessed by a CT scan within 3-month intervals or at any time as determined by the physicians.

Statistical analysis. OS was measured from the time of the diagnosis as incurable metastatic disease to the date of death or the last follow-up at December 31, 2013. All clinicopathological feathers were analyzed using the chi-square test or Fisher's exact test as indicated. The Kaplan–Meier method was used to estimate the probability of survival. The 95% confidence interval (CI) was also determined. The Cox proportional hazards regression model was used to calculate the hazard ratios and 95%CI in univariate and multivariate comparisons for every factor that might potentially affect OS. *p*-Values less than 0.05 were considered statistically significant, and all tests were two-tailed. All data analyses were performed using SPSS version 17.0 software (Chicago, IL, USA).

Results

Patients' clinicopathological factors. A total of 2734 patients were screened for enrollment. Among them, 2571 patients were excluded based on the inclusion and exclusion criteria, resulting in 163 patients being analyzed (Figure 1). The male:female ratio was 1:1.14. Among patients with *KRAS* mutations, the most frequently observed subtypes were G12D (36.2%), G12V (23.9%), and G13D (22.1%) (Table I). In addition, 48 (29.4%) out of 163 patients had a history of treatment with cetuximab-based regimens; the remaining 115 (70.6%) patients had never received cetuximab-based regimens.

As shown in Table I, there was no significant difference in gender, primary site, *KRAS*–mutation subtype, and treatment with systemic agents between the groups. However, patients that had received cetuximab were significantly younger than those who had never received cetuximab (median age of 53 years and 64 years, respectively). In addition, there was a trend that more patients treated with cetuximab were initially diagnosed with metastatic disease. Furthermore, whereas only 80.9% of patients that never used cetuximab received irinotecan during the follow-up period, 93.8% of patients treated with cetuximab had also received irinotecan during the follow-up period.

Out of the patients in the cetuximab-treated group, 20 (41.7%) received cetuximab as their first-line therapy, 5 (10.4%) as second-line treatment, and 18 (37.5%) as third-line therapy. Only one patient received cetuximab monotherapy.

Survival analysis by cetuximab treatment. Among all patients enrolled, 114 events were observed. The median OS for the study participants overall (N=163) was 27.4 months (95% CI=22.4-32.4 months). As shown in Figure 2A, the median

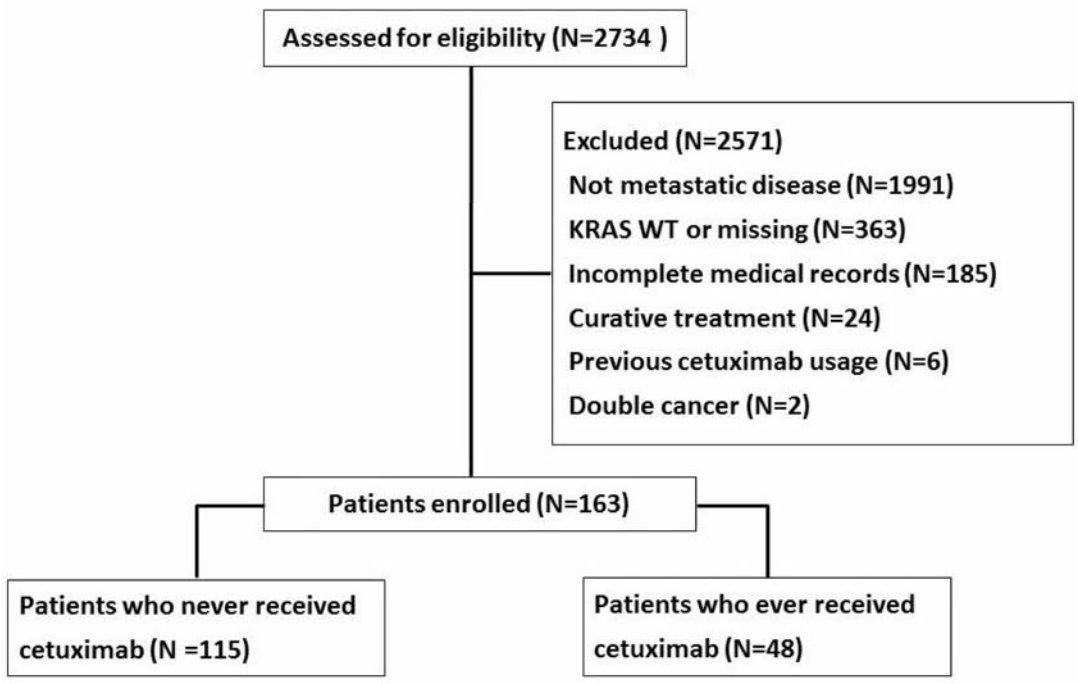

Figure 1. *Patient enrollment and exclusion.*

OS was 29.5 months (95% CI=18.4-40.6 months) in patients who had never used cetuximab-based regimens and 19.0 months (95% CI=9.6-28.4 months) in those who had a history of cetuximab-based treatment (p=0.040). Analysis of patients with all codon 12 mutations revealed that the median OS of those who had never used cetuximab-based regimens was significantly longer than those who had [32.0 months (95%CI=21.8-42.2 months) *versus* 17.5 months (95%CI=7.7-27.3 months), respectively; p=0.017) (Figure 2B).

Because there was only one patient with the *KRAS* G13C mutation, all patients with *KRAS* codon 13 mutations were analyzed as a whole. However, no differences were observed in patients with mCRC with KRAS codon 13 mutations. Specifically, the median OS was 25.8 months (95%CI= 5.5-46.1 months) in those who had never used cetuximab-based regimens and 27.4 months (95%CI= 10.2-44.5 months) in those treated with cetuximab-based regimens (p=0.890, Figure 2C).

Analysis of *KRAS*-mutation subtypes revealed that the median OS was significantly longer in patients with the *KRAS* G12V mutant tumors who had never used cetuximab-based regimens compared to those who had a history of cetuximab-based treatment [25.6 months (95%CI= 14.7-36.4 months) *versus* 12.6 months (95%CI= 7.0-18.2 months), respectively; (p=0.034) (Figure 2D). Although a similar trend was observed in patients with *KRAS* G12D-mutant tumors, it did not reach statistical significance (p=0.127).

Cox proportional hazards analysis. The Cox proportional hazard model was used to identify other potential confounding factors that might influence OS in patients with mCRC with *KRAS* mutations. The factors included gender (female *versus* male), age (<65 years *versus* ≥65 years), treatment with or without cetuximab, location of the tumor (distal *versus* proximal), *KRAS* mutation subtypes (codon 12 *versus* codon 13 mutations), chemotherapy or bevacizumab treatment, and initial stage of the disease (stage I-III *versus* stage IV). As shown in Table II, univariate and multivariate analyses revealed that the absence of cetuximab-based therapies was an independent prognostic factor for longer median OS in patients with mCRC with *KRAS* mutations.

Discussion

In the present study, we demonstrated that cetuximab application in patients with mCRC with certain *KRAS* mutations might not only be ineffective, but also detrimental. When we further analyzed specific *KRAS* mutations, this detrimental tendency was evident in patients with KRAS codon 12 mutant tumors, especially in patients with tKRAS G12V-mutant tumors. No such difference in OS was detected in patients with KRAS codon 13-mutant tumors. These results suggest that there was heterogeneity in mCRC response to mAbs to EGFR based upon specific *KRAS* mutation subtypes.

Table I. *Patients' clinical characteristics*.

Characteristic	All patients (%)[a]	Never-used cetuximab (%)[a]	Ever-used cetuximab (%)[a]	*p*-Value
All	163 (100%)	115 (70.6%)[d]	48 (29.4%)[d]	
Gender				
Female	90 (55.2%)	63 (54.8%)	27 (56.2%)	1.000
Male	73 (44.8%)	52 (45.2%)	21 (43.8%)	
Age (years)				
Median	61	64	53	
Range	24-87	24-87	28-80	
Age >65 years	61 (37.4%)	49 (42.6%)	12 (25.0%)	0.050
Primary site				0.258
Colon	113 (62.9%)	81 (70.4%)	32 (66.7%)	
Cecum	10 (6.1%)	8 (7.0%)	2 (4.2%)	
Ascending	33 (20.2%)	22 (19.1%)	11 (22.9%)	
Transverse	10 (6.1%)	9 (7.8%)	1 (2.1%)	
Descending	6 (3.7%)	6 (5.2%)	0 (0%)	
Sigmoid	54 (33.1%)	36 (31.3%)	18 (37.5%)	
Rectal	45 (27.6%)	32 (27.8%)	13 (27.1%)	
Other[b]	5 (3.1%)	2 (1.8%)	3 (6.3%)	
Initial stage				
IV	85 (52.1%)	54 (47.0%)	31 (64.6%)	0.058
I-III	78 (47.9%)	61 (53.0%)	17 (35.4%)	
Treatment[c]				
5-FU	163 (100.0%)	115 (100.0%)	48 (100.0%)	-
Oxaliplatin	138 (84.7%)	96 (83.5%)	42 (87.5%)	0.637
Irinotecan	138 (84.7%)	93 (80.9%)	45 (93.8%)	0.054
Bevacizumab	95 (58.3%)	65 (56.5%)	30 (62.5%)	0.601
KRAS mutation				0.645
G12D	59 (36.2%)	42 (36.5%)	17 (35.4%)	
G12V	39 (23.9%)	29 (25.2%)	10 (20.8%)	
G13D	36 (22.1%)	23 (20.0%)	13 (27.1%)	
G12A	9 (5.5%)	7 (6.1%)	2 (4.2%)	
G12C	11 (6.7%)	7 (6.1%)	4 (8.3%)	
G12S	7 (4.3%)	6 (5.2%)	1 (2.1%)	
G12R	1 (0.6%)	0 (0%)	1 (2.1%)	
G13C	1 (0.6%)	1 (0.9%)	0 (0%)	
Codon 12 mutation	126 (77.3%)	91 (79.1%)	35 (72.9%)	0.416
Codon 13 mutation	37 (22.7%)	24 (20.9%)	13 (27.1%)	

[a]The percentage is based on subgroup analysis. [b]Synchronous cancer. [c]Agents ever received during the follow-up period. [d]The percentage is based on all 163 cases.

There is great debate about the use of mAbs to EGFR in patients with *KRAS*-mutant mCRC. An extremely low response rate of 1.2% was observed in the CO. 17 trial, with only one responder out of a total of 81 patients with *KRAS* mutant tumors treated with cetuximab (26). In addition, inferior response rates and PFS for mCRC patients with *KRAS* mutations who received mAbs to EGFR plus chemotherapies compared to those who received cytotoxic agents alone have been reported in many prospective trials, including the CRYSTAL and PRIME trials (1, 5-8, 10, 23, 24), as well as many retrospective meta-analyses (21, 23, 24). In the OPUS trial, the median PFS for patients with *KRAS*-mutant tumors who received cetuximab plus FOLFOX-4 was only 5.5 months, which was significantly shorter than that of those who received FOLFOX-4 alone (8.6 months) (1, 3). However, the inferior response to mAbs to EGFR did not always translate into reduced median OS. In the CAIRO-2 trial, which included patients with mCRC who received first-line capcitabine/oxaliplatin/bevacizumab with/without cetuximab, subgroup analysis revealed that the median OS in patients that received capcitabine/ oxaliplatin/bevacizumab plus cetuximab was 17.2 months, compared to 24.9 months in patients who did not receive cetuximab (8). Our study provides additional support for the possibility that the median OS might be reduced under mAb to EGFR treatment in patients with mCRC with *KRAS* mutations.

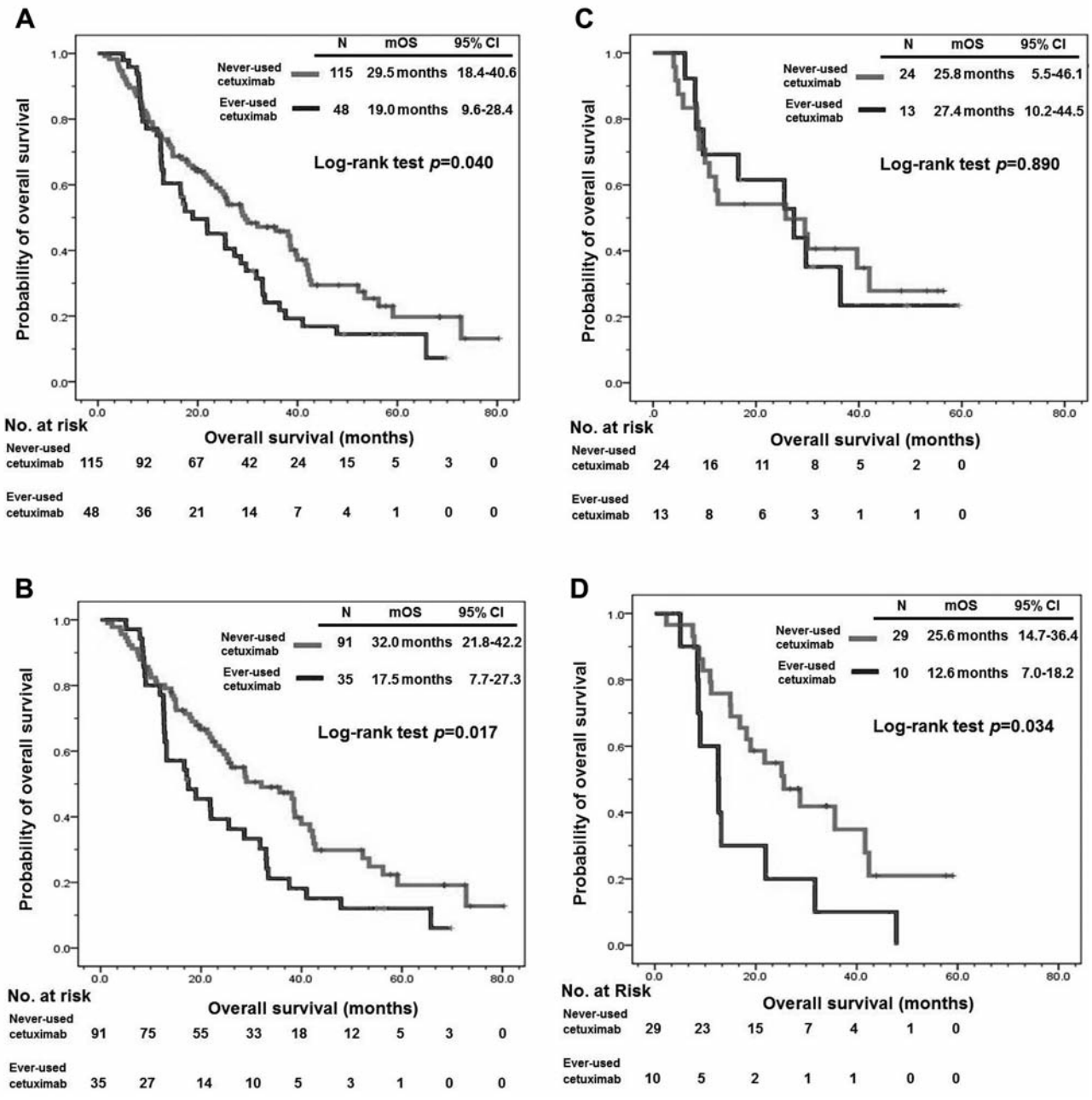

Figure 2. *Kaplan–Meier plots of overall survival by cetuximab use for the whole patient cohort (A), patients with KRAS codon 12 mutations (B), patients with KRAS codon 13 mutations (C), and patients with KRAS G12V mutation (D).*

We also sought to determine the possible causes of the conflicting results with respect to the effects of mAbs to EGFR on PFS and OS. One of the possible hypotheses is that different *KRAS* mutations lead to different response to EGFR mAbs. Some studies have suggested the possibility that tumors harboring *KRAS* mutations might be heterogeneously responsive to mAbs against EGFR and tumors with the G13D mutation might differ greatly from those with codon 12 mutations (12-16, 27, 28). In a pooled analysis, Peeters *et al*. reported that patients with *KRAS* G12A mutant tumors who received panitumumab had significantly shorter median OS as compared to those who did not receive panitumumab (18). In a meta-analysis by Mao *et al*. that included a total of 1,487 patients in 10

Table II. *Cox proportional hazards analysis for overall survival.*

	Univariate analysis			Multivariate analysis		
Variable	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Age						
>65 years	1.00			1.00		
≤65 years	0.81	0.55-1.20	0.30	0.92	0.61-1.38	0.67
Gender						
Male	1.00			1.00		
Female	0.79	0.54-1.14	0.21	0.76	0.52-1.11	0.16
KRAS mutation						
Codon 13	1.00			1.00		
Codon 12	1.05	0.67-1.64	0.83	1.07	0.67-1.73	0.77
Initial stage						
I-III	1.00			1.00		
IV	0.78	0.54-1.13	0.19	0.73	0.49-1.08	0.12
Primary site						
Distal[b]	1.00			1.00		
Proximal[c]	0.81	0.55-1.20	0.29	0.85	0.57-1.26	0.41
Cetuximab						
Ever-used	1.00			1.00		
Never-used	1.50	1.02-2.20	0.04[a]	1.54	1.03-2.30	0.04[a]
Bevacizumab						
Ever-used	1.00			1.00		
Never-used	1.12	0.77-1.63	0.56	1.07	0.73-1.58	0.73
Oxaliplatin						
Ever-used	1.00			1.00		
Never-used	0.57	0.33-0.96	0.03[a]	0.52	0.30-0.88	0.02[a]
Irinotecan						
Ever-used	1.00			1.00		
Never-used	1.06	0.62-1.80	0.83	0.90	0.46-1.75	0.75

CI, Confidence interval; HR, hazard ratio. [a]$p<0.05$. [b]Including the splenic flexure, descending colon, sigmoid colon, and rectum. [c]Including the cecum, ascending colon, and transverse colon.

studies, those with G13D-mutant tumors who received cetuximab had a significantly longer median OS compared to patients with other codon 12 mutations (19). Our study also indicated that mAbs against EGFR might not be effective in patients with *KRAS* codon 12-mutant tumors, which was not observed in those with codon 13 mutations. In fact, few studies mentioned above were analyzed according to the individual *KRAS*-mutant subtype. Therefore, this heterogeneity could potentially explain the conflicting results regarding the effects of cetuximab use on the PFS and OS because most previous clinical trials pooled the data on *KRAS*-mutant tumors rather than evaluating specific mutations.

The timing of cetuximab application in our group of patients was primarily either as first- or third-line therapy. In the GERCOR study, which was designed for patients with mCRC receiving either FOLFIRI followed by FOLFOX or FOLFOX followed by FOLFIRI, the median OS was 21.5 months in the former arm *versus* 20.6 months in the latter (29). Furthermore, many previous reports, including the CAIRO and MRC FOCUS trials, suggested that the sequence of treatment had no influence on patient survival in the pure chemotherapy era, and there is still no strong evidence for the timing of cetuximab usage in mCRC treatment (29-33). However, most meta-analyses and pooled analyses included patients that received cetuximab as first-line therapy (19, 20). Our study could clarify the role of the cetuximab in the treatment of mCRC with *KRAS* mutations based on samples in real clinical practice.

There were some limitations to our study. Firstly, this was a retrospective, single-center study that included patients with heterogeneous clinical conditions and treatment modalities. Although this study reflects the real-world practice, a further prospective trial is warranted. In addition, the size of the present study was not large enough to permit complex subgroup analysis, such as analyzing each *KRAS*-mutant subtype as well as the timing of cetuximab usage. Therefore, an additional study with a larger sample size is also needed. Thirdly, the clinicopathological differences between two groups, including the patients' age and the initial stage of the malignant disease, may interfere with the

interpretation of the results. Although we included these factors in the multivariate analysis, none of them had any clear influence on survival. Finally, in both univariate and multivariate analyses, there was a strong negative effect on median OS in patients who had never used oxaliplatin. As we might speculate, the definition of ever or never-use of oxaliplatin was confined to patients who were diagnosed as having unresectable mCRC. For patients who were initially diagnosed as having stage II or III CRC, they might have already received oxaliplatin in an adjuvant setting. Due to these confounding factors, the true effect of oxaliplatin on mCRC with *KRAS* mutations should be interpreted more carefully in larger-scale studies in the future.

In conclusion, this single-center, retrospective study demonstrated that cetuximab usage might not only be ineffective, but also detrimental in patients with mCRC with *KRAS*-mutant tumors, especially in patients with *KRAS* codon 12 mutations but not in patients with *KRAS* codon 13 mutations. Expanding the sample size for further specific analysis is needed before the initiation of prospective trials.

Conflicts of Interest

The Authors declare that they do not have any conflict of interest.

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