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Prospective analysis of *KRAS* wild-type patients with metastatic colorectal cancer using cetuximab plus FOLFIRI or FOLFOX4 treatment regimens

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ABSTRACT. Cetuximab, a monoclonal antibody targeting epidermal growth factor receptor, has proven to be efficient in the treatment of metastatic colorectal cancer. We made a prospective study of the efficacy and toxicities of cetuximab-combination first-line (FOLFOX4) versus second/third-line (FOLFIRI) chemotherapy in 98 *KRAS* wild-type patients who had metastatic colorectal cancer. Wild-type *KRAS* had been identified by direct sequencing. Associations between clinical response/progression-free survival/overall survival/toxicities and cetuximab-combination chemotherapy timing were evaluated. The overall response rate was significantly higher for first-line treatment than for second/third-line treatment (relative risk = 1.707, 95% confidence interval = 1.121-2.598). Both progression-free survival and overall survival indicated significantly longer survival of first-line treatment than second/third-line treatment patients. This study is a validation of a molecular analysis of *KRAS* wild-type status for the prediction of response to cetuximab-combination chemotherapy for metastatic colorectal cancer patients; its predictive role was less prominent in the second/third-line than in the first-line treatment patients.

Key words: Cetuximab; *KRAS*; Metastatic colorectal cancer; EGFR; 2nd- or 3rd-line setting; Wild-type

INTRODUCTION

In frequency of incidence of all cancers, colorectal cancers (CRC) rank fourth in men and third in women with approximately 1 million new cases in 2002 (9.4% of the world total), and 529,000 deaths due to CRC are reported around the world annually (Parkin et al., 2005). In Taiwan, CRC is one of the most common malignancies and is the third leading cause of cancer-related death. The incidence of CRC in Taiwan was 35.06/100,000 in 2004 and has been gradually approaching Western figures in recent decades. More than 10,500 new cases of CRC were diagnosed and more than 4100 Taiwanese died from CRC in 2007 (Department of Health, 2007).

The treatment of metastatic CRC (mCRC) has evolved significantly over the last decade. In the previous decade, significant improvements were made in response rates, progression-free survival (PFS), and overall survival (OS) of mCRC patients (Maindrault-Goebel et al., 2001; Teufel et al., 2004; Folprecht et al., 2006; Recchia et al., 2008). Because of the limited response obtained for patients with advanced CRC from first-line chemotherapy [5-fluorouracil (5-FU) modulated by leucovorin (LV)], other therapeutic agents with different mechanisms were obtained later such as infusional 5-FU/LV plus irinotecan regimen (FOLFIRI) or oxaliplatin plus infusional 5-FU/LV (FOLFOX) (Fakih, 2008). This prominent improvement is mainly due to the recent introduction of new combinations of chemotherapy and the new therapeutic agents targeting molecular events involved in colorectal carcinogenesis such as monoclonal antibody (mAb) against epidermal growth factor receptor (EGFR) or mAb against vascular endothelial growth factors.

The EGFR antibody, cetuximab (Erbitux®, ImClone Systems Inc., New York, NY, USA, and Bristol-Myers Squibb Co., Princeton, NJ, USA), induces synergistic antitumor activity when combined with chemotherapy. Cetuximab is a recombinant human/mouse chimeric EGFR immunoglobulin-G1 monoclonal antibody. Previous studies have shown that the benefits of the anti-EGFR mAb-cetuximab among patients with mCRC are limited to those who have colorectal tumor tissues with *KRAS* wild-type genes, and *KRAS* genes with mutation are essentially insensitive to EGFR inhibitors (Lievre et al., 2008; Van Cutsem et al., 2009; Yen et al., 2010). Several clinical trials have investigated the combination of cetuximab with FOLFIRI or FOLFOX chemotherapy for the first-line treatment of mCRC in Caucasians (Saltz et al., 2004; Lenz et al., 2006; Min et al., 2007; Tabernero et al., 2007; Arnold et al., 2008). While this regimen is undoubtedly active, these data were mainly from Western countries. The predictive role of *KRAS* wild-type in mCRC patients treated with cetuximab with FOLFIRI or FOLFOX chemotherapy as the first-line setting is well known. However, no relevant information regarding the combined approach of cetuximab with FOLFIRI or FOLFOX chemotherapy as the second/third-line therapy for mCRC patients has been found up to the present time.

Herein, the purpose of this study was to investigate the role of *KRAS* wild-type status in the prediction of clinical response and PFS/OS of mCRC patients using cetuximab-combination chemotherapy as the second/third-line setting in comparison with the first-line setting.

MATERIAL AND METHODS

Study population

From July 2007 through July 2010, we prospectively analyzed 98 histologically confirmed mCRC patients treated with cetuximab plus either FOLFIRI or FOLFOX-4 chemotherapy and for whom tumor DNA was available. All tumor tissues were identified to be *KRAS* wild-type status by DNA extraction and direct sequencing, using proteinase-K (Stratagene, La Jolla, CA, USA) digestion and the phenol/chloroform extraction procedure according to the method by Sambrook et al. (1989). The designed sequences of oligonucleotide primer for exons 2 and 3 of the *KRAS* and the operation procedure of direct sequencing were according to our previous study (Wang et al., 2003). An automated DNA electrophoresis system (Model 4200; LI-COR) with a laser diode emission at 785 nm and

fluorescence direction between 815 and 835 nm was used to detect and analyze the sequencing ladders. To be eligible for the study, mCRC patients with measurable lesions by computed tomographic scan were enrolled. Patients were required to be at least 18 years of age with a life expectancy of 3 months, and have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2. Patients with other malignant diseases in their medical history were excluded. All patients were required to have adequate hematologic, renal, and liver function data and no concurrent severe or life-threatening illness. The patients' clinical characteristics are listed in Table 1.

Chemotherapy regimen

The patients were divided into two groups based on the different setting (first-line or second/third-line). Patients received biweekly cetuximab at a dose of 500 mg/m^2 in a 2-h infusion, followed by FOLFIRI or FOLFOX-4 chemotherapy on day 1 of a 14-day cycle. FOLFIRI was conducted comprising 180 mg/m^2 irinotecan as a 2-h infusion on day 1, 400 mg/m^2 LV as a 2-h infusion concurrently with irinotecan on day 1, 400 mg/m^2 5-FU as an intravenous (*iv*) bolus infusion followed by 2400 mg/m^2 infusion *iv* over a 46-h period, which was repeated every 2 weeks. FOLFOX-4 was conducted comprising 85 mg/m^2 oxaliplatin as a 2-h infusion on day 1, 200 mg/m^2 LV as a 2-h infusion concurrently with oxaliplatin on day 1, followed by a bolus of 400 mg/m^2 5-FU and a continuous infusion of 600 mg/m^2 5-FU over 22 h, which was repeated every 2 weeks. For tumor staging, initial work-up included general history and physical examination, routine blood cell count, biochemistry, and serum carcinoembryonic antigen (CEA) level examination. For further image study, chest X-ray, abdominal echo or abdominal computed tomography (CT) scan and magnetic resonance imaging (MRI) were performed. Bone scan or positron emission tomography (PET) was performed selectively for those who showed suspicious findings on CT or MRI or where specific sites of metastases were suspected.

Assessment of chemotherapy response

The major objectives of this study were to assess the safety and efficacy of these two settings (first-line or second/third-line) of cetuximab-combination chemotherapy. The assessment of toxicities was based on National Cancer Institute Common Toxicity Criteria (version 3.0) (http://ctep.cancer.gov/reporting/ctc.html; accessed in April 2010). The time for the first-response assessment with CT or other imaging study was typically performed 2-3 months after the first assessment. Patient responses were classified according to Response Evaluation Criteria in Solid Tumors (RECIST) (Therasse et al., 2000). A complete response was defined as the disappearance of all target lesions of cancer in response to treatment. A partial response was defined as at least 30% decrease in the sum of the longest diameter of metastatic lesions, with no evidence of new lesions. A progressive disease was defined as at least a 20% increase in the sum of the longest diameter of target lesions, taking as a reference the smallest sum of the longest diameter recorded before the patient started receiving treatment, and it could also be defined if identification of one or more new lesions was made. A stable disease was defined as neither having sufficient shrinkage to qualify for a partial response nor a sufficient increase to qualify for progressive disease. We report here the best response, which was

defined as the best response recorded by an independent investigator. Also, the PFS and OS were compared between the two groups.

Follow-up

The clinical records for each patient of this study were retrospectively reviewed. The characteristics of the patients being recorded include age, gender, metastatic sites, the different setting of cetuximab-combination chemotherapy, and observed toxicities encountered after the chemotherapy. Safety assessment and laboratory tests were performed biweekly. Courses of chemotherapy were continued in the presence of an absolute neutrophil count ≥1500/μL and platelet count ≥100,000/μL and recovery of any extra-hematological toxicity. Otherwise, for patients with grade 3 or more severe hematologic toxicities, treatment was postponed for one or two weeks until recovery and restarted when it had reduced to grade 2. Both settings were continued until one of the following occurred: progressive disease, unacceptable adverse effects, the patient refused further treatment with any cetuximab-combination chemotherapy, or the patient was lost to follow-up. The median follow-up period was 26 months (range, 4-42 months). This study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital, and was not supported by any commercial company.

Statistical analysis

All data were analyzed using the Statistical Package for the Social Sciences version 12.0 software (SPSS Inc., Chicago, IL, USA). Many descriptive variables of patient characteristics included were analyzed by the Fisher exact test used to compare response in two different groups. Using the calculator for survival probability (the Kaplan-Meier method), PFS was calculated as the period combined with time interval of month from the first day of cetuximab treatment to the date of tumor progression, to the date of death from any cause, or to the date of the last follow-up. OS was also followed-up like the PFS as the period from the first day of cetuximab treatment until death from any cause or until the date of the last follow-up. Typically, the log-rank test is used to compare the survival distributions of PFS and OS. A probability of less than 0.05 was considered to be statistically significant.

RESULTS

The characteristics of these 98 mCRC patients are summarized in Table 1. All 98 patients were classified into two groups according to the two different settings of cetuximab-combination chemotherapy (first-line setting as group A; second/third-line setting as group B). The mean ± SD age was 58.8 ± 7.8 years in group A (range, 39 to 76) and 58.5 ± 9.2 years in group B (range, 40 to 80). Within the two different groups, there were 25 males and 23 females in group A, and 26 males and 24 females in group B. Among the 48 patients of group A, there were 32 patients (66.7%) with primary tumors located in the colon and 16 patients (33.3%) with tumors located in the rectum. Among the 50 patients of group B, 34 patients (68%) had primary tumors located in the colon and 16 patients (32%) had tumors located in the rectum. The main site of metastases was the liver (50% in group A, 42% in group B) followed by the lung (22.9% in group A and 24% in group B). In addition, 16.7 % in group A and 22% in group B had metastases in more than one site.

Table 1. Patient characteristics.

Variables	Group A (N = 48)	Group B (N = 50)	P
Age (years, mean ± SD)	58.8 ± 7.8	58.5 ± 9.2	0.382
Gender			
male	25 (52.1%)	26 (52.0%)	0.993
female	23 (47.9%)	24 (48.0%)	
Primary site			
colon	32 (66.7%)	34 (68.0%)	0.888
rectum	16 (33.3%)	16 (32.0%)	
Site of metastases			
liver only	24 (50.0%)	21 (42.0%)	0.955
lung only	11 (22.9%)	12 (24.0%)	
peritoneum only	4 (8.3%)	3 (6.0%)	
ovarian only	2 (4.2%)	2 (4.0%)	
nonregional lymph node only	1 (2.1%)	1 (2.0%)	
≥2 sites	8 (16.7%)	11 (22.0%)	
ECOG performance status			
0	28 (58.3%)	21 (42.0%)	0.175
1	15 (31.3%)	18 (36.0%)	
2	5 (10.4%)	11 (22.0%)	
Chemotherapy regimen			
cetuximab + FOLFOX	27 (56.3%)	27 (54.0%)	0.823
cetuximab + FOLFIRI	21 (43.7%)	23 (46.0%)	

Data are reported as number with percent in parentheses. Group A = cetuximab as first-line setting; Group B = cetuximab as second/third-line setting; ECOG = Eastern Cooperative Oncology Group.

A total of 48 patients in group A who underwent first-line cetuximab-combination chemotherapy and 50 patients in group B who underwent second/third-line cetuximab-combination chemotherapy were assessed for responses. The main objective responses of these patients are summarized in Table 2. Among the 48 patients in group A, a complete response was observed in one case (2.1%), partial response was observed in 29 cases (60.4%), stable disease was observed in 9 cases (18.8%), and progressive disease was observed in 9 cases (18.8%). For the 50 patients in group B, none had complete response, 18 patients (36%) had partial response, 15 patients (30%) had stable disease, and 17 patients (34%) had progressive disease. Overall, the response rate (complete response plus partial response) reached 62.5% (30/48) in group A and 36% (18/50) in group B. Comparing groups A and B, we observed a statistically significant positive association with the better response rate of group A (RR = 1.707, 95%CI = 1.121-2.598; P = 0.009). In this prospective cohort of mCRC patients, the presence of the first-line cetuximab was statistically positively associated with tumor response, while group B was not associated with a rise in tumor sensitivities for cetuximab.

Table 2. Efficacy of patients receiving cetuximab-combined FOXFOX4 or FOLFIRI chemotherapy.

	Group A (N = 48)	Group B (N = 50)	P
Responder	30 (62.5%)	18 (36.0%)	
Complete response	1 (2.1%)	0 (0.0%)	
Partial response	29 (60.4%)	18 (36.0%)	0.037
Non-responder	18 (37.5%)	32 (64.0%)	
Stable disease	9 (18.8%)	15 (30.0%)	
Progressive disease	9 (18.8%)	17 (34.0%)	

Data are reported as number with percent in parentheses. Group A = cetuximab as first-line setting; Group B = cetuximab as second/third-line setting.

Figure 1 shows the PFS and OS Kaplan-Meier curve of these two different dosage groups. The median time to progression was 10.0 months in group A and 6.0 months in group B. PFS had a statistically significant difference between the two groups after analysis (P = 0.001), and OS also showed a significant longer overall survival in group A than in group B (P = 0.0022). The efficacy showed that first-line setting cetuximab-combination chemotherapy was better than the efficacy of the second/third-line setting.

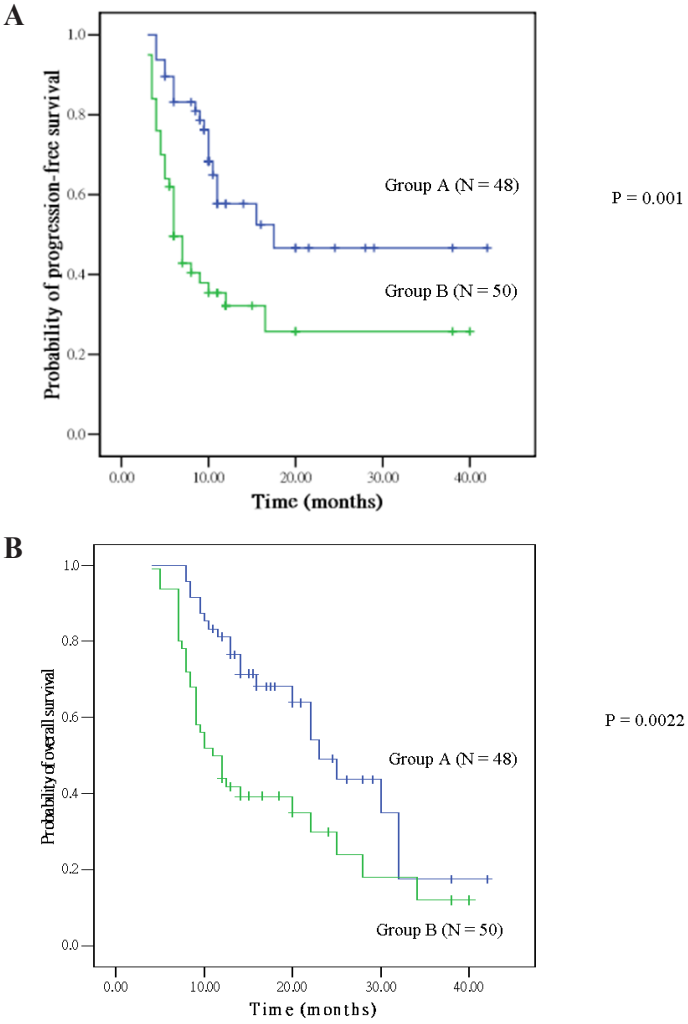

Figure 1. The progression-free survival and overall survival of the metastatic colorectal cancer patients treated with cetuximab-combination chemotherapy as the first-line (group A) *vs* the second/third-line (group B) setting. **A.** Analysis of progression-free survival of the metastatic colorectal cancer patients treated with cetuximab-combination chemotherapy as the first-line (group A) *vs* the second/third-line (group B) setting. The progression-free survival of group A was statistically significantly longer than in group B (P = 0.001). **B.** Analysis of overall survival of the metastatic colorectal cancer patients treated with cetuximab-combination chemotherapy as the first-line (group A) *vs* the second/third-line (group B) setting. The overall survival of group A was statistically significantly longer than in group B (P = 0.0022).

As shown in Table 3, neutropenia was the most common grade 3 or 4 adverse event of group A (14.6%, 7/48), consistent with the profile of group B (14%, 7/50) (P = 0.934). Among other hematological side effects including anemia and thrombocytopenia, there were four (8.3%) cases suffering from anemia or thrombocytopenia in group A and four cases (8%) in group B (P = 1.000). In all cases, all these hematological side effects were usually short-lived and were rarely complicated. No patients experienced these side events leading to cessation of therapy. Neither grade 4 myelosuppression nor severe infusional anaphylactic reactions were found in either group.

Table 3. Grade 3/4 toxicities of patients receiving cetuximab-combined FOXFOX4 or FOLFIRI chemotherapy.

	Group A (N = 48)	Group B (N = 50)	P
Skin rash	7 (14.6%)	6 (12.0%)	0.706
Neutropenia	7 (14.6%)	7 (14.0%)	0.934
Anemia	4 (8.3%)	4 (8.0%)	1.000
Thrombocytopenia	4 (8.3%)	4 (8.0%)	1.000
Diarrhea	5 (10.4%)	6 (12.0%)	0.804
Stomatitis	5 (10.4%)	6 (12.0%)	0.804
Elevated AST/ALT	4 (8.3%)	5 (10.0%)	1.000
Paronychia	7 (14.6%)	6 (12.0%)	0.706
Alopecia	2 (4.2%)	2 (4.0%)	1.000
Fatigue/asthenia	6 (12.5%)	7 (14.0%)	0.827

Data are reported as number with percent in parentheses. Group A = cetuximab as first-line setting; Group B = cetuximab as second/third-line setting; AST = aspartate transaminase; ALT = alanine transaminase.

Rates of toxicity-related gastrointestinal side effects were similar across these two groups and always could be easily treated. However, grade 3 or 4 diarrhea occurred in 5 patients (10.4%) in group A and 6 patients (12%) in group B. In groups A and B, 10.4% (5/48) and 12% (6/50), respectively, complained of grade 3 stomatitis. All these gastrointestinal side effects could be easily controlled or corrected by antiemetics, antidiarrheal agents and intravenous fluid supplement. In both groups, no therapies were discontinuous consequently. Drug-related serious liver dysfunctions were reported in both groups. Elevated aspartate transaminase (AST) and alanine transaminase (ALT) were found in four patients (8.3%) in group A. In group B, the hepatic toxicities were similar and 5 patients (10%) had elevated AST and elevated ALT. It seems that there was no significant correlation between different line settings and abnormal liver function tests (P = 1.000). Concerning other specific side events, there were also similar events across group A and B. No treatment-related deaths occurred. Overall, the safety of the first-line setting cetuximab-combination chemotherapy was consistent with the second/third-line setting chemotherapy without meaningful increase in toxicity, and both were proven to be well tolerated.

DISCUSSION

In general, rates of incidence of CRC are increasing rapidly in various countries where overall risk was formerly low (especially in Japan and also elsewhere in Asia) (Parkin et al., 2005), and the same trend is occurring in Taiwan. The therapeutic mainstay for CRC is the 5-FU/LV regimen. Until recently, the standard systemic treatment of mCRC had been directed to FOLFOX or FOLFIRI (Fakih, 2008). In recent decades, advances in the understanding of the tumor biology from CRC have led to the identification of important cellular processes in-

volved in the pathogenesis, and drugs, which interfere with these critical pathways, are known as target therapy (Reidy and Saltz, 2007). EGFR is involved in signaling pathways that affect cellular growth, differentiation, proliferation, and programmed cell death, and is a transmembrane glycoprotein that is often overexpressed in CRC (Hemming et al., 1992; Midgley et al., 2009). Cetuximab, a chimeric monoclonal immunoglobulin G1 antibody that binds to the extracellular domain of the EGFR and inhibits the EGFR, has been found to be effective alone and in combination with irinotecan in patients with mCRC as second/third-line treatment of mCRC in patients who are refractory to irinotecan-based chemotherapy (Cunningham et al., 2004; Midgley et al., 2009). In clinical practice, although some mCRC patients who carried wild-type *KRAS* have poor response to the initial standard FOLFOX or FOLFIRI treatment, they still have good response after the addition of cetuximab later. Consequently, the predictive value of the wild-type *KRAS* among mCRC patients refractory to first-line or second-line chemotherapy is needed before the clinical implication.

Our present investigation shows that response rate and the PFS between the first-line and second/third-line setting groups were significantly different in group A: one patient (2.1%) had complete response and 29 patients (60.4%) had partial response resulting in an overall response rate of 62.5% (30/48). In group B, no patient had complete response and 18 patients (36%) had partial response resulting in a significantly poorer overall response rate of 36% (18/50). Furthermore, the PFS/OS of group A was statistically significantly longer than in group B. Our response rate and PFS/OS of group B were compatible with the results of several previous studies from Western countries (Pfeiffer et al., 2007; Martin-Martorell et al., 2008). In group A, the response rate and PFS/OS were similar when compared to the results of several previous studies from Western countries (Folprecht et al., 2006), but were significantly better than group B, and those findings are vital for mCRC patients for the administration of the first-line or second/third-line cetuximab-combination chemotherapy. mCRC patients with *KRAS* wild-type status will have a better predictable response, PFS and OS when cetuximab-combination chemotherapy is used as the first-line therapy compared to the second/third-line setting.

The mechanism of drug resistance of cancer cells to oxaliplatin or irinotecan could contribute to the poor response of the second/third-line therapy. Most recently, oxaliplatin has been approved in the USA as a first-line therapy in combination with 5-FU for the treatment of mCRC. Resistance to platinum agents has been attributed to enhanced tolerance to platinum DNA adducts, decreased drug accumulation and enhanced DNA repair (Bleiberg et al., 1996). Proteins of the nucleotide excision repair (NER) pathway, in particular, are thought to play a key role in the repair of DNA damage caused by platinum compounds. Hence, the possible mechanism of the second/third-line setting cetuximab-combination chemotherapy presenting poorer response than the first-line setting needs to be further investigated. Changes in the DNA-repair function and the rate of inactivation of the administrated chemotherapeutic compound may also determine drug efficacy in the tumor tissue. Irinotecan causes S-phase-specific cell killing by poisoning topoisomerase I (Topo I) in the cell. Several studies have been done to uncover possible mechanisms for the cellular resistance to this agent, such as its resistance in human small-cell and non-small cell lines with low carboxylesterase expression (van Ark-Otte et al., 1998) and the repair of irinotecan-induced DNA damage coupled with RNA transcription (Liu et al., 2000). P-glycoprotein and multidrug resistance-associated protein family of transporters play important roles in the efflux and active excretion of irinotecan and decreasing the intracellular level (Loe et al., 1996). As Topo I is the cellular target, in irinotecan-resistant

human colon cancer cell lines rendered resistant by stepwise, continuous treatment with irinotecan, the total activity of Topo I was shown to be reduced (Giovanella et al., 1989). Moreover, gene alterations in the downstream of KRAS/ERK/MAPK-signaling cascade, which activates transcription factors critical for angiogenesis, proliferation, apoptosis, differentiation, and metastasis (Fang and Richardson, 2005), may probably lead to a less predictive value of *KRAS* wild-type status in cetuximab-combination chemotherapy as a second/third-line therapy in mCRC patients. However, further studies are mandatory to demonstrate this hypothesis. There is one limitation of the present study. No doubt, the treatment in first-line works better than in second-line but this seems to be an obvious observation. However, the predictive role of *KRAS* wild-type status in cetuximab-combination chemotherapy in the second/third-line setting compared to the first-line setting remains a crucial issue in clinical practice.

The adverse events reported here are similar for two different settings. A low percentage of patients experienced grade three or four neutropenia/anemia/thrombocytopenia or gastrointestinal side events in our investigation. The rate and severity of patients developing mild to moderate gastrointestinal toxicities were lower than previous reports (Kallen et al., 2000; Giacchetti et al., 2000). A low percentage of patients with elevated AST/ALT, fatigue, paronychia, and alopecia were found in our investigation. In both groups, no patient lost further treatment because of the adverse events encountered, and in terms of toxicity, the first-line and second/third-line cetuximab-combination chemotherapy were both proven to be well tolerated.

In conclusion, it is feasible to assume that *KRAS* wild-type status is a responsive predictor in different settings of cetuximab-combination chemotherapy for mCRC patients; however, in clinical implication, mCRC patients are more likely to benefit from the first-line setting than the second/third-line setting using *KRAS* wild-type status as a molecular predictor.

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