

Oncologic Outcomes in Metastatic Colorectal Cancer with Regorafenib with FOLFIRI as a Third- or Fourth-Line Setting^{1,2,3}



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Abstract

BACKGROUND: To evaluate the efficacy and toxicities of regorafenib plus irinotecan, dose-escalated on the basis of *uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1)* genotyping, in previously heavily treated metastatic colorectal cancer (mCRC) and the prognostic values of EGFR expression, *KRAS* mutations, and tumor sidedness. **METHODS:** Forty-one patients with mCRC with disease progression after treatment with fluoropyrimidines, oxaliplatin, irinotecan, anti-VEGF, and anti-EGFR MoAbs were subjected to *UGT1A1* genotyping and received regorafenib combined with FOLFIRI with dose-escalated irinotecan. **RESULTS:** The median follow-up period was 10.0 months (1.3–23.5 months). The overall disease control rate was 58.5%, whereas the median progression-free survival (PFS) and overall survival (OS) were 6.0 months and 12.0 months, respectively. *KRAS* mutations were significantly associated with positive EGFR expression ($P = .026$). *KRAS* mutations significantly correlated with a shorter OS than *KRAS* wild-type (6.0 vs. 14.4 months, $P = .014$) but had no significant association with PFS. Positive EGFR expression had an inverse correlation with PFS (2.5 vs. 14.0 months, $P = .039$) and OS (9.6 vs. 19.7 months, $P = .044$). Moreover, left-sided tumors associated with superior PFS (2.0 vs. 7.0 months, $P < .0001$)

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and OS (4.0 vs. 13.0 months, $P < .0001$), and tumor sidedness was an independent prognostic factor by the multivariate analysis. **CONCLUSION:** Regorafenib and FOLFIRI concomitant therapy with dose-escalated irinotecan seemed to be potentially practicable with satisfactory oncological results. *KRAS* mutations and EGFR expression might be predictors of poor oncological outcomes; however, left-sided mCRCs would be more beneficial for concomitant regorafenib and FOLFIRI therapy.

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the fourth leading cause of cancer-related deaths worldwide; an estimated 1.4 million newly diagnosed cases and nearly 700,000 deaths were reported in 2012 [1]. Metastases coincide with approximately one-fourth of the CRC cases at the time of diagnosis (synchronous), and the initially local diseases progress to metastases in approximately 40% of the rest of the cases (metachronous), accounting for up to 60% of CRC cases that require treatment for metastatic diseases [2].

Metastatic CRCs (mCRCs) are principally managed with chemotherapy composed of fluoropyrimidines and either oxaliplatin or irinotecan combined with monoclonal antibodies (MoAbs) targeting vascular endothelial growth factor (VEGF) or epidermal growth factor receptor (EGFR) if *RAS* is wild type. Regorafenib is an oral multikinase inhibitor that targets and inhibits receptor tyrosine kinases (RTKs) in the Ras/Raf/MEK/ERK pathway, which participates in the signaling of oncogenesis, angiogenesis, and cancer proliferation and metastasis [3]. Regorafenib monotherapy serves as a salvage treatment for progressive mCRCs that are unresponsive to fluoropyrimidines, irinotecan, oxaliplatin, and anti-VEGF and anti-EGFR MoAbs. In the global CORRECT study, which compared regorafenib monotherapy and placebo in previously treated mCRC, regorafenib monotherapy yielded a significantly superior disease control rate (DCR), progression-free survival (PFS), and overall survival (OS) compared with placebo in previously treated mCRC (41% vs.15%, $P < .0001$; 1.9 vs.1.7 months, $P < .0001$; 6.4 vs.5.0 months, $P = .0052$, respectively) [4]. Another CONCUR trial in the Asian population revealed similar significant differences in DCR, PFS, and OS between regorafenib monotherapy and placebo (51.5% vs. 7.4%, $P < .0001$; 3.2 vs.1.7 months, $P < .0001$; 8.8 vs.6.3 months; $P = .00016$, respectively) [5]. For grade >3 adverse events (AEs) of regorafenib, such as hand-foot skin reaction (HFSR), anemia, thrombocytopenia, and small intestine hemorrhage, daily doses of regorafenib from 160 mg to 120 mg are usually reduced, and the oncological outcomes may be comparable [6]. Furthermore, *uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1)* genotyping is approved by the Food and Drug Administration of the United States for predicting irinotecan-related severe diarrhea and neutropenia [7] because UGT1A1 protein is the main enzyme responsible for glucuronidation of the active metabolite of irinotecan 7-ethyl-10-hydroxycamptothecin (SN-38) and therefore metabolizes and detoxifies irinotecan [8]. However, *UGT1A1* genotyping also predicts the maximum dose of irinotecan that can be tolerated and enables dose adjustment of irinotecan [9–11]. We previously introduced dose-reduced regorafenib combined with dose-adjusted irinotecan based on individual *UGT1A1* genotyping along with 5-fluorouracil (5-FU) plus leucovorin (FOLFIRI regimen) with satisfactory oncological results [12].

The right-sided colon differs from the left-sided colon and rectum both in embryological origin and in blood supply, where the right-sided colon arises from midgut, blood supply comes from superior mesentery vessels, and the left-sided colon and rectum are hindgut structures supplied from inferior mesentery vessels. The prognostic impact of tumor sidedness also differs in that right-sided mCRCs have poor prognosis and have limited benefit from systemic chemo- and targeted therapy. The CALGB/SWOG 80405 study demonstrated that *KRAS* wild-type metastatic right-sided colon cancers had a shorter median OS than metastatic left-sided colon cancers (19.4 months vs. 33.3 months, hazard ratio [HR] = 1.55, 95% confidence interval [CI] = 1.32-1.82, $P < .0001$). In those who received bevacizumab, OS was 24.2 months for right-sided mCRCs and 31.4 months for left-sided mCRCs (HR = 1.32, 95% CI = 1.05-1.65, $P = .01$), whereas OS was 16.7 months for right-sided tumors and 36 months for left-sided tumors treated with cetuximab (HR = 1.87, 95% CI = 1.48-2.32, $P < .0001$). In addition, bevacizumab showed better outcomes than cetuximab in right-sided tumors regardless of *KRAS* status. On the contrary, *KRAS* wild-type left-sided mCRCs had more benefit from cetuximab than bevacizumab [13].

KRAS mutations have been clearly identified as a predictor marker for response to anti-EGFR MoAbs in patients with mCRC, and anti-EGFR MoAbs are therefore exclusively administered for *KRAS* wild-type tumors [14–20]. Although EGFR expression may be a negative prognostic factor in CRC after curative resection and associated with resistance to chemotherapy [21–24], increased EGFR gene copy number has a prominent correlation with prognosis in mCRC treated with first-line anti-EGFR MoAbs [25–28]. Primary or acquired resistance to anti-EGFR MoAbs may manifest in previously treated mCRC with mutations in EGFR downstream signaling pathways: PI3K/Akt/mTOR and Ras/Raf/MEK/ERK, which regorafenib targets. The roles of EGFR expression and *KRAS* status in mCRC previously treated with chemotherapy and anti-EGFR or anti-VEGF MoAbs remain unclear. Thus, the present study aimed to analyze the prognostic value of EGFR expression, *KRAS* mutation, and tumor sidedness in patients with mCRC treated with regorafenib and FOLFIRI as a third- or fourth-line setting.

Materials and Methods

Participants

This retrospective study recruited 41 patients with progressing mCRC who were previously treated with FOLFOX, FOLFIRI, anti-VEGF MoAb, and anti-EGFR MoAb if *KRAS* wild-type tumors were identified, between October 2013 and June 2018 in a single institute. The protocol was approved by the institutional ethics committees and conducted in accordance with the 1964 Declaration of Helsinki

(2008 revision). The study was registered at clinicaltrials.gov under the identification code ID NCT03698253 (www.clinicaltrials.gov/ct2/show/NCT03698253) and the trial name Metastatic Colorectal Cancer Treated With Regorafenib and FOLFIRI.

Immunohistochemical Analysis for EGFR Expression

Formalin-fixed and paraffin-embedded tissue blocks were cut into 3- μ m sections and deparaffinized, rehydrated, and autoclaved at 121°C for 5 minutes in Target Retrieval solution (Dako, Glostrup, Denmark) with pH 6.0 to retrieve antigens. Endogenous peroxidase was blocked using 3% hydrogen peroxide for 5 minutes at room temperature. After the sections were washed with a Tris buffer solution, they were incubated with EGFR for 1 hour at room temperature. Then, the DAKO REAL EnVision Detection System-HRP (DAKO, Glostrup, Denmark) was applied for 30 minutes at room temperature. Finally, the sections were incubated in 3',3'-diaminobenzidine for 5 minutes followed by counterstaining with Mayer's hematoxylin. Dehydration was performed through two changes of 95% ethanol and two changes of 100% ethanol, and the samples were cleared in three changes of xylene and subsequently mounted. Negative controls were obtained by replacing the primary antibody with nonimmune serum. EGFR immunoreactivity was evaluated by two independent researchers who were blind to patient outcome. The expression patterns of EGFR were determined in a semiquantitative manner through light microscopy. EGFR immunoreactivity (membrane staining) was categorized according to the presence of tumor cell staining and staining intensity. The intensity of EGFR immunoreactivity was scored using a three-tier system as follows [29]: 1+ (weak intensity), 2+ (moderate intensity), and 3+ (strong intensity). A negative EGFR expression was defined as the absence of membrane staining above the background in all tumor cells, whereas a positive EGFR expression was defined as complete or incomplete immunohistochemical membrane staining of tumor cells with intensities of 1+, 2+, or 3+.

DNA Extraction and Direct Sequencing of KRAS

Genomic DNA was isolated from frozen primary CRC tissues through proteinase-K (Stratagene, La Jolla, CA) digestion and phenol/chloroform extraction. The designed sequences of oligonucleotide primers for exons 2 and 3 of *KRAS* and the operational procedure of direct sequencing were based on those of our previous study [30].

Study Design

For each patient, regorafenib and FOLFIRI with dose-adjusted irinotecan according to *UGT1A1* genotyping were administered. Because grade ≥ 3 hand-foot syndrome developed frequently in patients receiving oral regorafenib at 160 mg/day (21 days at a 7-day interval), the dose was adjusted to 120 mg/day. If grade ≥ 3 regorafenib-induced AEs such as hand-foot syndrome still developed, regorafenib was discontinued until the AEs subsided. Furthermore, according to our previous clinical results [9], patients with *UGT1A1**1/*1 and *1/*28 genotypes were initially given a standard dose of 180 mg/m² irinotecan, and those with the *UGT1A1**28/*28 genotype were given 120 mg/m² of irinotecan. Irinotecan was administered for over 2 hours on day 1 followed by 5-FU (2800 mg/m² intravenously infused for over 46 hours in a 2-week cycle). For all the patients, the irinotecan dose was increased by 30 mg/m² up to a maximal dose of 260 mg/m² every two cycles until grade ≥ 3 AEs or severe AEs

(SAEs) of irinotecan developed, following which the dose was reverted to and maintained at the previously tolerated level.

The treatment response was radiologically assessed every 2 months through computed tomography, magnetic resonance imaging, or positron emission tomography. Objective responses were classified according to the Response Evaluation Criteria in Solid Tumors [31], and optimal treatment responses were recorded. Common Terminology Criteria for Adverse Events version 3.0 [32] was used for evaluating treatment-associated AEs. Treatment with regorafenib plus dose-escalated irinotecan was stopped if progressive disease or grade ≥ 3 AEs occurred.

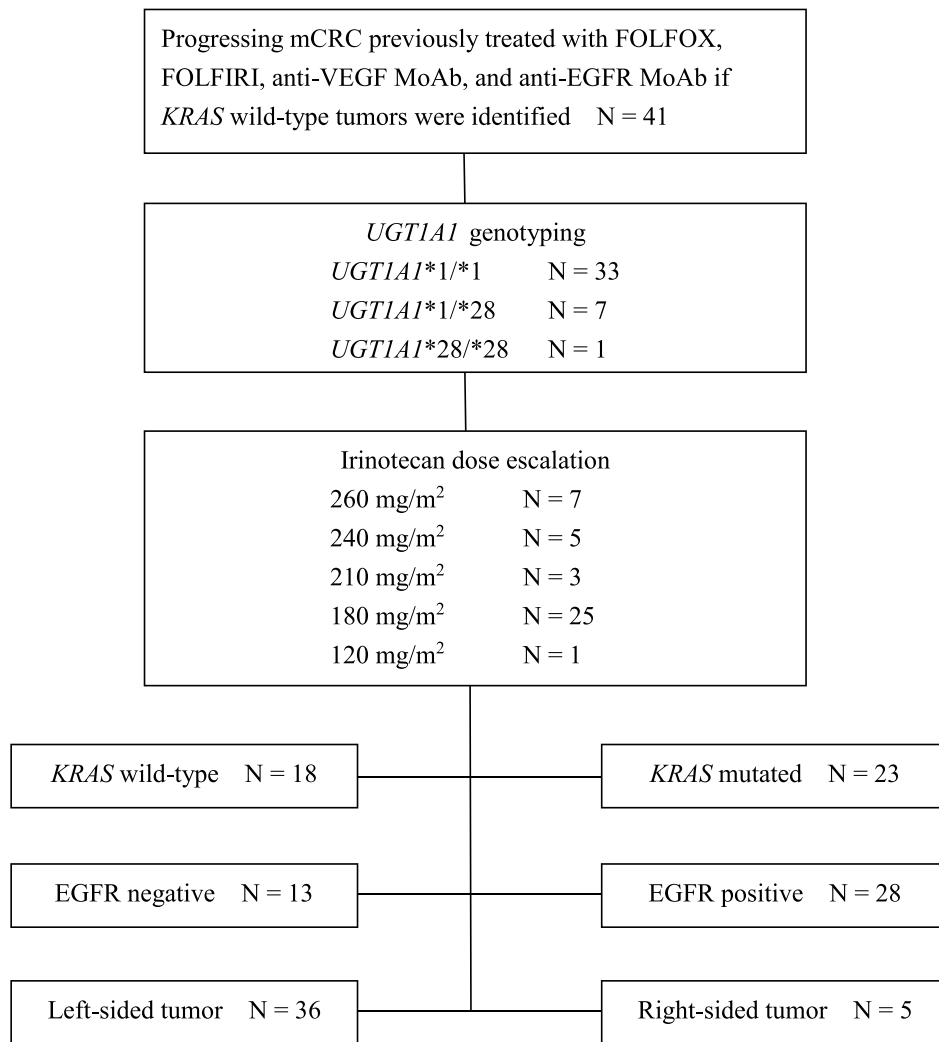
Statistical Analysis

Categorical data were compared using the Mann-Whitney *U* test or Kruskal-Wallis test. The Fisher's exact test was used to compare dichotomous variables, and the Pearson chi-square test was used to analyze nominal variables. The means were compared using the two-sample test and analysis of variance or linear regression, as appropriate. However, for all aforementioned inferential analysis methods, the center effect was not considered in comparing the two treatments. Therefore, analysis of variance incorporating the center effect and Cochran-Mantel-Haenszel test stratified by the center effect were applied to replace the two-sample *t* test and Fisher's exact test. For efficacy analyses and part of the safety analyses (including laboratory data and vital sign data), considering the effect of baseline data on the endpoints, analysis of covariance was applied to compare the mean of one treatment with that of another, with their respective baseline values as covariates. PFS and OS were calculated using the Kaplan-Meier method. PFS was defined as the time from treatment initiation until the first radiological evidence of progression, whereas OS was defined as the time from treatment initiation until death from any cause. Statistical analyses were conducted using SPSS 20.0 (SPSS, Chicago, IL). A *P* value less than .05 was considered statistically significant.

Results

The study scheme is shown in [Figure 1](#). In summary, the median follow-up period was 10.0 months (1.3-23.5 months). The overall disease control rate was 58.5%, whereas the median PFS and OS were 6.0 months and 12.0 months, respectively. *KRAS* mutations were significantly associated with positive EGFR expression (*P* = .026). *KRAS* mutations significantly correlated with a shorter OS than *KRAS* wild-type (*P* = .014) but had no significant association with PFS (*P* = .117). Positive EGFR expression had an inverse correlation with PFS (*P* = .027) and OS (*P* = .021). Moreover, left-sided tumors associated with superior PFS (*P* < .0001) and OS (*P* < .0001), and tumor sidedness was an independent prognostic factor by the multivariate analysis.

Demographic data of the studied patients are summarized in [Table 1](#). A total of 41 patients were enrolled in the present study, with a median age of 61 years (36-85 years) and male predominance (61.0% vs. 39.0%). The most common site of metastasis was the liver (69.5%) followed by the lung (56.1%), peritoneum (17.1%), brain (2.4%), and neck lymph nodes (2.4%). Of the 41 patients, 17 presented two or more sites of metastasis (41.5%), and the remaining patients presented a single site of metastasis. *KRAS* mutation was found in 23 patients (56.1%), and positive EGFR expression was noted in 28 patients (68.3%). The number of *UGT1A1**1/*1 genotypes was overwhelming in patients with the highest



mCRC: metastatic colorectal cancer; VEGF: vascular endothelial growth factor; EGFR: epidermal growth factor receptor; MoAb: monoclonal antibody

Figure 1. The study scheme of the present study.

administered irinotecan dose of 260 mg/m² (180-260 mg/m²). The corresponding irinotecan dose was 120 mg/m² for the only patient with the *UGT1A1**28/*28 genotype. All the patients with *KRAS* mutated type CRC were previously treated with bevacizumab and FOLFIRI (standard irinotecan dose of 180 mg/m²) in the first-line setting, followed by FOLFOX6 regimen. For patients with *KRAS* wild-type tumors, cetuximab plus FOLFIRI was often first administered in patients with *KRAS* wild-type tumors; this was followed by FOLFOX6 and bevacizumab plus FOLFIRI in the third-line setting (irinotecan dose was 180 mg/m²). An additional FOLFOXIRI regimen was administered to two of these *KRAS* wild-type patients before the reimbursement of regorafenib by Taiwan National Health Insurance as the fourth-line treatment. Consequently, regorafenib plus FOLFIRI with dose-escalated irinotecan served as the third-line salvage treatment for *KRAS* mutated type tumors and the fourth- or fifth-line treatment for *KRAS* wild-type tumors. All the treatments were substituted only when diseases progressed or patients were intolerant.

The median follow-up period was 10.0 months (1.3-23.5 months). The patients were followed up until June 2018 or their death. The median duration of regorafenib treatment was 6.0 months (1.3-23.5 months). The most frequently encountered grade ≥3 AE was HFSR (*n* = 13, 31.7%), followed by mucositis (*n* = 7, 17.1%), neutropenia (*n* = 4, 18.2%), diarrhea (*n* = 5, 12.2%), and fatigue (*n* = 3, 7.3%). The majority of the patients had stable disease (SD) (*n* = 20, 48.8%), 4 patients (9.8%) had partial response (PR), and 17 (41.5%) had progressive disease (PD), with an overall DCR of 58.5%. The only patient with the *UGT1A1**28/*28 genotype and positive EGFR expression with *KRAS* mutated cancer received only one cycle of regorafenib plus the initial irinotecan dose of 120 mg/m²; however, she developed grade 3 mucositis and subsequently died. The overall median PFS and OS were 6.0 months (95% CI: 0.2-11.8) and 12.0 months (95% CI: 7.4-16.6), respectively (Figure 2).

A summary of *KRAS* status analysis is shown in Table 2. *KRAS* mutation had a significant correlation with positive EGFR expression

Table 1. Demographic Data of Study Patients

Clinical Characteristics	Number of Cases (%)
Sex	
Male	25 (61.0)
Female	16 (39.0)
Median age (years)	61.0 (36-85)
Site of metastasis	
Liver	27 (65.9)
Lung	23 (56.1)
Peritoneum	7 (17.1)
Brain	1 (2.4)
Neck lymph nodes	1(2.4)
Number of sites of metastasis	
1	24 (58.5)
2	17 (41.5)
<i>KRAS</i> status	
Wild type	18 (43.9)
Mutated	23 (56.1)
EGFR status	
Positive	28 (68.3)
Negative	13 (31.7)
Tumor sidedness	
Left sided	36 (87.8)
Right sided	5 (12.2)
<i>UGT1A1</i> status	
*1/*1	33 (80.5)
*1/*28	7 (17.1)
*28/*28	1 (2.4)
Irinotecan dose (mg/m ²)	
260	7 (17.1)
240	5 (12.2)
210	3 (7.3)
180	25 (61.0)
120	1 (2.4)
Lines of systemic therapy	
3rd	23 (56.1)
4th	16 (39.0)
5th	2 (4.9)
≥Grade 3 AEs	
Hand-foot skin reaction	13 (31.7)
Mucositis	7 (17.1)
Neutropenia	4 (18.2)
Diarrhea	5 (12.2)
Fatigue	3 (7.3)
Best objective response	
PR	4 (9.8)
SD	20 (48.8)
PD	17 (41.5)
DCR	24 (58.5)

EGFR, epidermal growth factor receptor; PR, partial response; SD, stable disease; PD, progressive disease; DCR, disease control rate; AEs, adverse events.

($P = .026$) and significant inverse correlation with grade ≥ 3 AEs ($P = .019$). By contrast, as listed in Table 3, positive EGFR expression was significantly associated with the poorer best objective response ($P = .027$) and DCR ($P = .021$). Table 4 shows a summary of analysis for tumor sidedness. Difference of *UGT1A1* genotype was associated significantly with tumor sidedness, that the only *UGT1A1**28/*28 genotype presented at right-sided tumor, whereas all *UGT1A1**1/*28 genotypes located at left-sided tumors ($P = .017$). Right-sided tumors had significantly worse response ($P = .018$), and all right-sided tumors were considered progressive disease. Consequently, right-sided tumors had zero DCR compared to 66.7% in left-sided ones ($P = .008$). *KRAS* wild-type mCRC was significantly correlated with a longer OS (14.4 vs. 6.0 months, 95% CI: 8.7-20.1 vs. 4.5-7.5, $P = .014$; HR = 0.40, 95% CI: 0.18-0.86) but had no correlation with PFS (9.0 vs. 3.5 months, 95% CI: 2.9-15.1 vs. 0-7.3, $P = .117$; HR = 0.57, 95% CI: 0.28-1.19, Figure 3). Moreover, negative EGFR expression had a significant positive effect

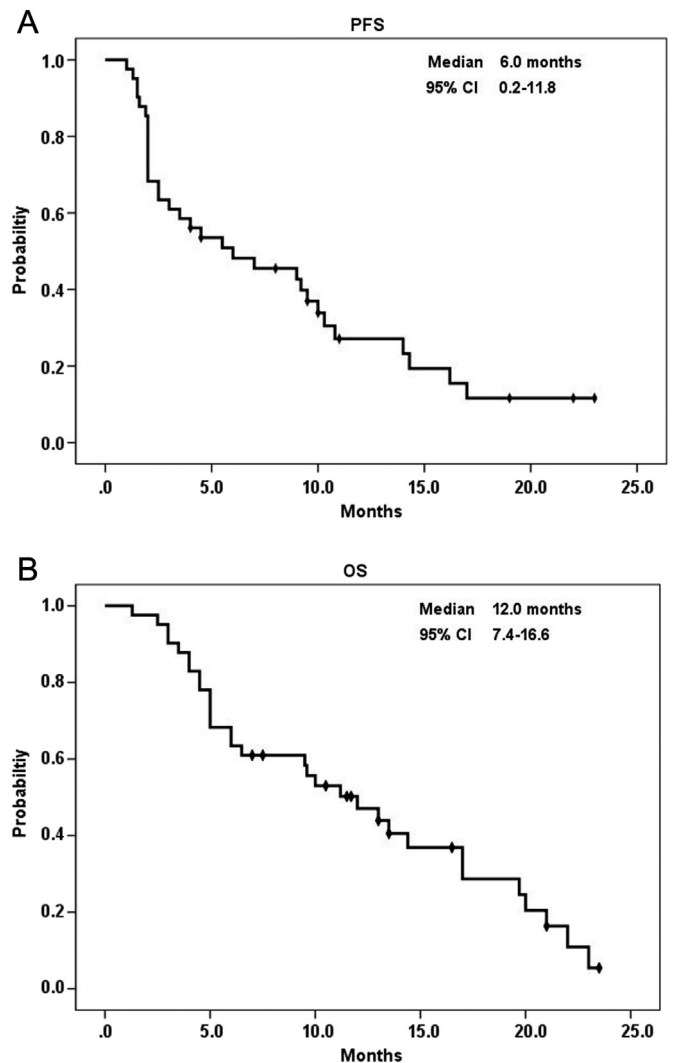


Figure 2. Kaplan-Meier survival analysis of all patients with metastatic colorectal cancer. (A) Progression-free survival and (B) overall survival.

on both PFS (14.0 vs. 2.5 months, 95% CI: 7.1-20.9 vs. 1.5-3.5, $P = .039$; HR = 0.44, 95% CI: 0.20-1.01) and OS (19.7 vs. 9.6 months, 95% CI: 7.1-32.3 vs. 4.2-15.0, $P = .044$; HR = 0.43, 95% CI: 0.18-1.02, Figure 4). Of utmost important, left-sided tumors were associated with a superior PFS (7.0 vs. 2.0 months, 95% CI: 1.9-12.0 vs. -, $P < .0001$; HR = 0.17, 95% CI: 0.06-0.52) and OS (13.0 vs. 4.0 months, 95% CI: 2.9-5.1 vs. 8.9-17.1, $P < .0001$; HR = 0.18, 95% CI: 0.06-0.53) to right-sided tumors as well (Figure 5). Furthermore, tumor sidedness remained an independent prognostic factor in a multivariate analysis adjusted with *KRAS* and EGFR status for PFS ($P = .010$, HR = 0.22, 95% CI: 0.07-0.69) and OS ($P = .004$, HR = 0.20, 95% CI: 0.07-0.59; Tables 5 and 6).

Discussion

Irinotecan must be converted by a carboxylesterase to SN-38, which is actively cytotoxic and detoxified by the glucuronidation activity of UGT, primarily by the *UGT1A1* isoenzyme; consequently, the *UGT1A1* genotype represents the development of drug-associated AEs. Because of the decreased capacity of the *UGT1A1**28 allele to metabolize SN-38, patients with homozygous *UGT1A1**28/*28 are

Table 2. KRAS Status Subgroup Analysis of the Clinical Features of Study Patients

	KRAS Wild Type (N = 18)	KRAS Mutated (N = 23)	P
Sex, N (%)			.987
Male	11 (61.1)	14 (60.9)	
Female	7 (38.9)	9 (30.1)	
Median age (range), years	63 (36-85)	61 (37-82)	.651
EGFR status, N (%)			.026
Positive	9 (50.0)	19 (82.6)	
Negative	9 (50.0)	4 (17.4)	
Tumor sidedness			.363
Left side	17 (94.4)	19 (82.6)	
Right side	1 (5.6)	4 (17.4)	
UGT1A1 status			.421
*1/*1	16 (88.9)	17 (73.9)	
*1/*28	2 (11.1)	5 (21.7)	
*28/*28	0 (0)	1 (4.3)	
Irinotecan dose, N (%), mg/m ²			.375
260	5 (27.8)	2 (8.7)	
240	3 (16.7)	2 (8.7)	
210	1 (5.6)	2 (8.7)	
180	9 (50.0)	16 (69.6)	
120	0 (0.0)	1 (4.3)	
Best objective response, N (%)			.190
PR	3 (16.7)	1 (4.3)	
SD	10 (55.6)	10 (43.5)	
PD	5 (27.8)	12 (52.2)	
DCR, N (%)	13 (72.2)	11 (47.8)	.116
AEs			.019
Grade ≥3	15 (83.3)	11 (47.8)	
Grade <3	3 (16.7)	12 (52.2)	

EGFR, epidermal growth factor receptor; PR, partial response; SD, stable disease; PD, progressive disease; DCR, disease control rate; AEs, adverse events.

more vulnerable to irinotecan; thus, a low initial dose of irinotecan should be considered. A genotype-directed dose-determination study on irinotecan dose escalation in first-line FOLFIRI for mCRC conducted by Marcellino et al. reported that homozygous *UGT1A1**28/*28 genotype patients developed irinotecan-associated SAEs more frequently [33]. Patients with heterozygous polymorphism of the *UGT1A1* promoter (i.e., *UGT1A1**1/*28) undergo intermediate glucuronidation activity of *UGT1A1* and are considered to be at an increased risk of irinotecan toxicity. On the contrary, clinical presentations in patients with *UGT1A1**1/*28 vary individually, but these patients generally tolerate the recommended initial irinotecan dose of 180 mg/m² [34]. However, patients with the homozygous *UGT1A1**1/*1 genotype are more resistant to irinotecan-associated AEs and could tolerate an irinotecan dose as high as 260 mg/m² in previous observational studies [12,35]. As the result, for patient safety and treatment efficacy of irinotecan dose-escalated FOLFIRI, *UGT1A1* genotyping was carried out before treatment was initiated. In the present and aforementioned studies, an initial irinotecan dose of 180 mg/m² for the *UGT1A1**1/*1 and *UGT1A1**1/*28 and 120 mg/m² for the *UGT1A1**28/*28 genotypes was infused and subsequently increased by 30 mg/m² every two cycles. The irinotecan dose was reverted to the last tolerated dose if any irinotecan-related grade ≥3 AEs or SAEs developed. The final dose of irinotecan could be escalated to 210-260 mg/m² in 36.6% of the treated patients, and 61.0% of the patients discontinued irinotecan use at an initial dose of 180 mg/m² because of disease progression and received a substitute treatment. In the present study, the only patient with homozygous *UGT1A1**28/*28 tolerated an initial irinotecan dose of 120 mg/m²; however, grade 3 mucositis developed in this patient and might be associated with regorafenib. The irinotecan-associated grade ≥3 AEs that were

Table 3. EGFR Status Subgroup Analysis of the Clinical Features of Study Patients

	EGFR Negative (N = 13)	EGFR Positive (N = 28)	P
Sex, N (%)			.460
Male	9 (69.2)	16 (57.1)	
Female	4 (30.8)	12 (42.9)	
Median age (range), years	66 (51-85)	57 (36-82)	.147
KRAS status, N (%)			.026
Wild type	9 (69.2)	9 (32.1)	
Mutated	4 (30.8)	19 (67.9)	
Tumor sidedness			1.000
Left side	12 (92.3)	24 (85.7)	
Right side	1 (7.7)	4 (14.3)	
UGT1A1 status			.765
6/6	11 (84.6)	22 (78.6)	
6/7	2 (15.4)	5 (17.9)	
7/7	0 (0.0)	1 (3.6)	
Irinotecan dose, N (%), mg/m ²			.880
260	3 (23.1)	4 (14.3)	
240	2 (15.4)	3 (10.7)	
210	1 (7.7)	2 (7.1)	
180	7 (53.8)	18 (64.3)	
120	0 (0.0)	1 (3.6)	
Best objective response, N (%)			.027
PR	3 (23.1)	1 (3.6)	
SD	8 (61.5)	12 (42.9)	
PD	2 (15.4)	15 (53.6)	
DCR, N (%)	11 (84.6)	13 (46.4)	.021
AEs			.055
Grade ≥3	11 (84.6)	15 (46.4)	
Grade <3	2 (15.4)	13 (53.6)	

EGFR, epidermal growth factor receptor; PR, partial response; SD, stable disease; PD, progressive disease; DCR, disease control rate; AEs, adverse events.

encountered, such as neutropenia (18.2%) and diarrhea (12.2%), were acceptable; however, Li et al. revealed that the first-line fixed irinotecan dose of 180 mg/m² for mCRC resulted in a higher

Table 4. Tumor Sidedness Subgroup Analysis of the Clinical Features of Study Patients

	Left side (N = 36)	Right side (N = 5)	P
Sex, N (%)			.362
Male	23 (63.9)	2 (40.0)	
Female	13 (36.1)	3 (60.0)	
Median age (range), years	60.5 (36-85)	64.0 (40-72)	.311
KRAS status, N (%)			.363
Wild-type	17 (47.2)	1 (20.0)	
Mutated	19 (52.8)	4 (80.0)	
EGFR status, N (%)			1.000
Positive	24 (66.7)	4 (80.0)	
Negative	12 (33.3)	1 (20.0)	
UGT1A1 status			.017
*1/*1	29 (80.6)	4 (80.0)	
*1/*28	7 (19.4)	0 (0)	
*28/*28	0 (0)	1 (20.0)	
Irinotecan dose, N (%), mg/m ²			.064
260	7 (19.4)	0 (0)	
240	4 (11.1)	1 (20.0)	
210	3 (8.3)	0 (0)	
180	22 (61.1)	3 (60.0)	
120	0 (0.0)	1 (20.0)	
Best objective response, N (%)			.018
PR	4 (11.1)	0 (0)	
SD	20 (55.6)	0 (0)	
PD	12 (33.3)	5 (100.0)	
DCR, N (%)	24 (66.7)	0 (0)	.008
AEs			.336
≥ grade 3	24 (66.7)	2 (40.0)	
< grade 3	12 (33.3)	3 (60.0)	

ERFR: epidermal growth factor receptor; PR: partial response; SD: stable disease; PD: progressive disease; DCR: disease control rate; AEs: adverse events.

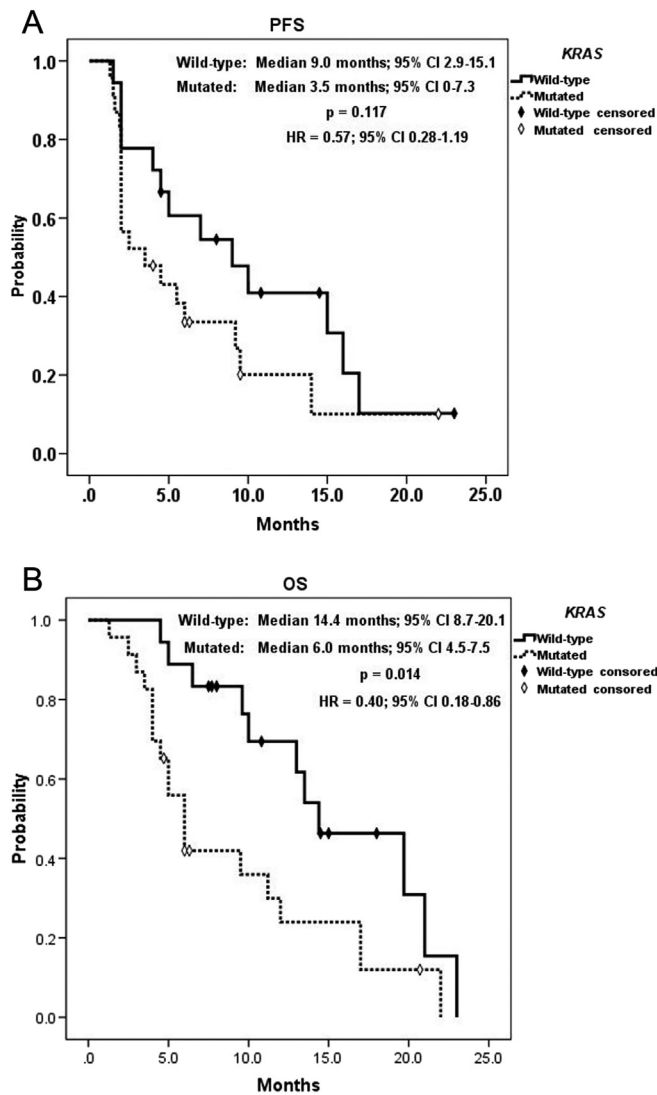


Figure 3. Kaplan-Meier survival analysis of *KRAS* subgroups. (A) Progression-free survival and (B) overall survival.

incidence of severe diarrhea in patients with single-allele or two-allele variants of *UGT1A1*6/*28*, without differences in severe neutropenia in patients with different alleles [36]. The *UGT1A1*6* allele, with a higher incidence in Asian populations, should be analyzed in a future study in addition to *UGT1A1*1* and **28* alleles that may further reduce irinotecan-related AEs through dose adjustment.

Regorafenib is a small molecule that targets and blocks RTKs, including EGFR, VEGF receptor, fibroblast growth factor receptor, platelet-derived growth factor receptor, RET, and KIT, which participate in oncogenesis, angiogenesis, and cell proliferation in the tumor microenvironment, consequently exhibiting antineoplastic capacity [3]. Because regorafenib inhibits multiple pathways, it induces various AEs, including HFSR, hypertension, thrombocytopenia, gastrointestinal bleeding, proteinuria, diarrhea, mucositis, and hepatotoxicity. HFSR is the most commonly encountered grade ≥ 3 AE associated with regorafenib, and it leads to dose reduction or interruption of treatment [4], which may impair the efficacy of regorafenib. Moreover, the incidence of HFSR is more frequent in the Asian population [37], and modification of the dosing schedule from

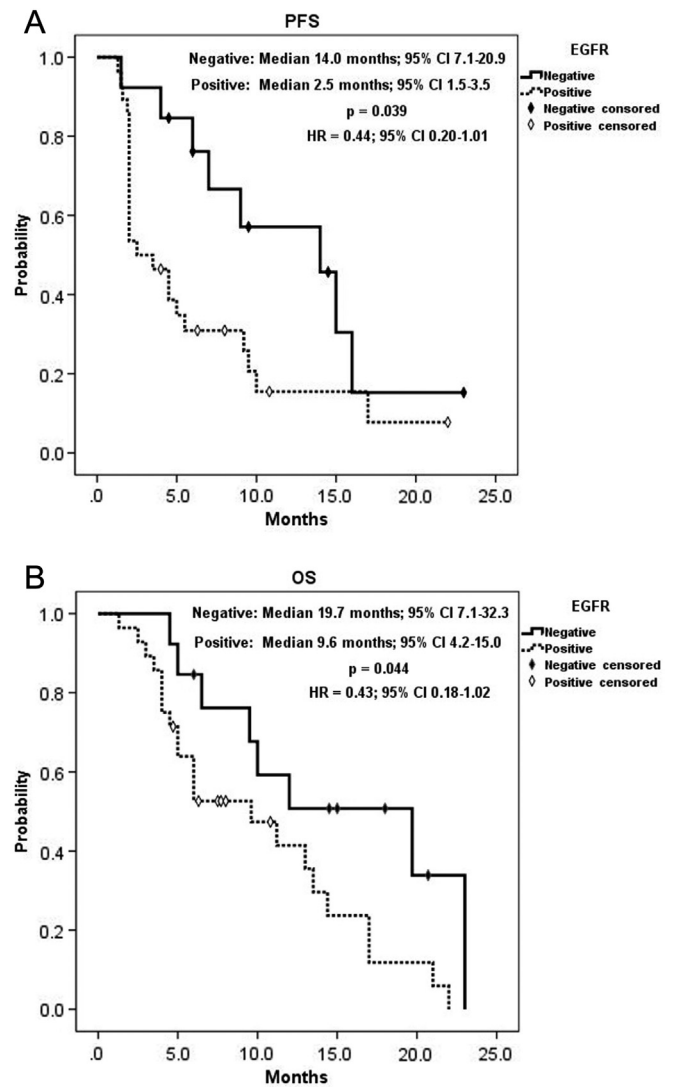


Figure 4. Kaplan-Meier survival analysis of EGFR expression subgroups. (A) Progression-free survival and (B) overall survival.

160 mg once daily for 3 weeks in a 4-week cycle to 120 mg daily for 4 weeks with the same accumulated dose of 3360 mg in a single cycle still yielded high rates of grade 3 HFSR, which recovered earlier than with the original dosing schedule though [12]. A dose reduction from 160 to 120 mg for 3 weeks in a 4-week cycle greatly reduced the occurrence of grade 3 HFSR (75% vs. 16.7%) with comparable DCR (60% vs. 58.3%) in a Japanese study [6]. The incidence of grade 3 HFSR was 31.7%, which generally recovered within 2 weeks after regorafenib stopped, with a dosing schedule of 120 mg daily for 4 weeks combined with FOLFIRI with dose-escalated irinotecan, and the oncological outcomes were favorable. Consequently, adjustment of the irinotecan dose based on *UGT1A1* genotyping optimizes the oncological efficacy with balanced toxicities for individuals, and reintroduction of irinotecan with dose escalation may have a synergic effect on regorafenib. Moreover, in a preclinical xenograft study on regorafenib and the potential combination therapy, a combination of regorafenib and irinotecan significantly delayed tumor growth after extended treatment in four xenograft models [38]. Consequently, we intend to conduct a multicenter, 2:1 randomized, controlled clinical trial with two parallel arms to compare regorafenib monotherapy and

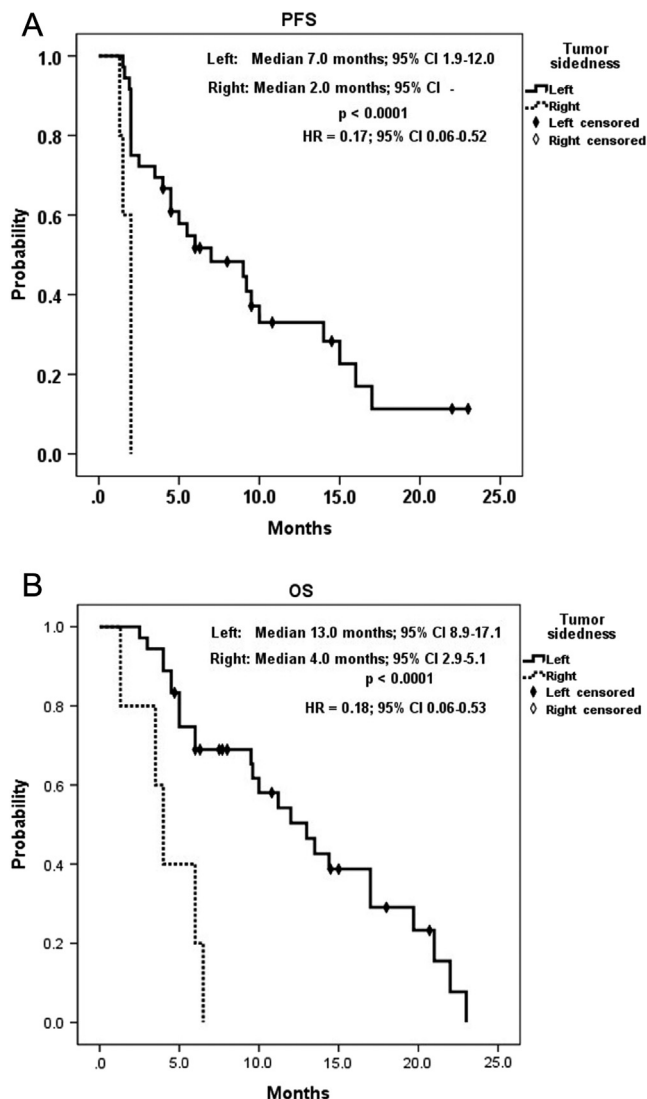


Figure 5. Kaplan-Meier survival analysis of tumor sidedness subgroups. (A) Progression-free survival and (B) overall survival.

regorafenib combined with FOLFIRI with dose-escalated irinotecan for previously treated mCRC according to the current results.

KRAS mutation has been well documented as a predictor of tumor resistance to anti-EGFR MoAbs, and anti-EGFR MoAbs are recommended to be administered restrictively for *KRAS* wild-type mCRC [19,20]. However, the responses to anti-EGFR MoAbs are variable in *KRAS* wild-type cancers. Alternations of EGFR and the downstream effectors of EGFR-activated PI3K/Akt/mTOR and Ras/Raf/MEK/ERK pathways contribute to such heterogeneous clinical outcomes. *KRAS*, *NRAS*, and *BRAF* mutations are associated with primary resistance to anti-EGFR MoAbs and are negative prognostic

Table 5. Univariate and Multivariate Progression-free Survival Analysis

Variable	Univariate (HR)	P	95% CI	Multivariate (HR)	P	95% CI
Tumor sidedness (left-sided)	0.17	.002	0.06-0.52	0.22	.010	0.07-0.69
<i>KRAS</i> (wild-type)	0.57	.136	0.28-1.19	0.80	.591	0.36-1.78
EGFR (negative)	0.44	.052	0.20-1.01	0.52	.144	0.22-1.25

HR: hazard ratio; CI: confidence interval; ERFR: epidermal growth factor receptor.

Table 6. Univariate and Multivariate Overall Survival Analysis

Variable	Univariate (HR)	P	95% CI	Multivariate (HR)	P	95% CI
Tumor sidedness (left-sided)	0.18	.002	0.06-0.53	0.20	.004	0.07-0.59
<i>KRAS</i> (wild-type)	0.40	.020	0.18-0.86	0.51	.117	0.22-1.18
EGFR (negative)	0.43	.054	0.18-1.02	0.54	.182	0.22-1.34

HR: hazard ratio; CI: confidence interval; ERFR: epidermal growth factor receptor.

factors [39–41]. *PI3KCA* gene mutations play roles in refractory activities; moreover, *PI3KCA* mutations are significantly correlated with a low response rate to cetuximab plus chemotherapy [42,43]. In addition, increased EGFR copy number [25,27,28], expression of EGFR ligands (amphiregulin, heparin-binding epidermal growth factor, transforming growth factor- α , and epiregulin) [26,44,45], and expression of phosphatase and tensin homologue (PTEN) [25,46,47], which is a downstream inhibitor of the EGFR pathway, are associated with more favorable response and survival rates, whereas HER2/MET overexpression is associated with poorer response and survival in anti-EGFR MoAb therapy [48–50]. However, most of the aforementioned studies have been conducted in the setting of anti-EGFR MoAbs as first- or second-line treatments, and investigations of biomarkers and regorafenib in anti-EGFR MoAbs refractory cases are rare. The multikinase inhibitory capacities of regorafenib overcome the molecular heterogeneity of the EGFR pathway and salvage those who developed primary or acquired resistance to anti-EGFR MoAbs. In the present study, positive EGFR expression correlated significantly to poorer DCR, PFS, and OS, and EGFR expression was a negative prognostic factor, similar to the role of EGFR expression in CRC not treated with anti-EGFR MoAbs. Moreover, similar PFS and OS in EGFR-positive subgroup with regorafenib monotherapy in CORRECT study reflected poor response to FOLFIRI and limited oncological benefit from FOLFIRI for EGFR-positive tumors. Consistent with the REBECCA study [51], unfavorable OS was significantly associated with *KRAS* mutations, with no relationship between PFS and *KRAS* mutations in the present study. Notably, patients with *KRAS* wild-type tumors presented a higher incidence of grade ≥ 3 AEs; this finding may be a clinical predictor for survival. EGFR expression with primary or acquired resistance to anti-EGFR MoAbs, in which EGFR-activated PI3K/Akt/mTOR and Ras/Raf/MEK/ERK pathways are not blocked but activated instead, is contributory to response differences in EGFR subgroup. On the other hand, presence of *KRAS* mutations indicates permanent “turned on” of Ras/Raf/MEK/ERK pathway. Regorafenib targets receptor tyrosine kinases (e.g., vascular endothelial growth factor receptor [VEGFR], platelet-derived growth factor receptor- β [PDGFR- β], fibroblast growth factor receptor [FGFR], and tyrosine kinase with immunoglobulin and epidermal growth factor homology domain [TIE2]) as well as intracellular kinases RAF1 and BRAF. The last two (ie. RAF1 and BRAF) are downstream effectors of RAS and regorafenib therefore blocks Ras/Raf/MEK/ERK pathway with *KRAS* mutations that results in similar PFS in the *KRAS* status subgroup though, options of treatments after disease relapse are limited in tumors with *KRAS* mutations and lead to unfavorable OS consequently.

CRC is a heterogeneous disease that right-sided colon differs from left-sided colon and rectum not only in embryological origin and anatomic location and blood supply but also in molecular and morphological alternations. According to the Consensus Molecular

Subtype (CMS) Consortium, CMS1 (microsatellite instability [MSI]-immune) tumors were frequently diagnosed in females with right-sided tumors and accounted for 14% of CRC [52]. CMS1 tumors are highlighted by hypermutated, defective DNA mismatch repair, MSI, *MLH1* silencing by promoter hypermethylation, CpG island methylation phenotype (CIMP), high *BRAF* V600E mutation rate, and immune infiltration in the tumor microenvironment. CMS1 tumors were associated with worse survival after relapse, consistent with studies exhibiting poor prognosis in mCRC with MSI and *BRAF* V600E mutation [53–55]. Immune checkpoint blockade therapy with immune checkpoint inhibitors to modulate immunogenicity of CMS1 tumors is recently introduced [56,57]. On the other hand, CMS2 (canonical) subtype was predominant in left-sided lesions [52,58]. CMS2 tumors are characterized by high frequency of DNA somatic copy number alternation (SCNA), typically initial loss of tumor suppressor *APC* gene followed by an activating mutation of *KRAS* and loss of *TP53*, which in turn activate WNT and MYC pathway. CMS2 subtype of CRC arises from canonical adenoma-carcinoma sequence and had superior survival after relapse and larger proportion of long-term survivors [52]. The prospective, observational CORRELATE study, in which patients with mCRC were treated with regorafenib monotherapy, demonstrated that tumor sidedness had no significant differences in both median PFS (2.8 months, 95% CI: 2.6–2.9 for left-sided tumors vs. 2.7 months, 95% CI: 2.5–3.1 for right-sided tumors) and median OS (7.4 months, 95% CI: 6.7–8.0 for left-sided tumors vs. 8.2 months, 95% CI: 6.6–9.3 for right-sided tumors) [59]. In comparison to the present study, regorafenib plus irinotecan dose-escalated FOLFIRI had similar outcomes in right-sided mCRCs, but PFS and OS were much better in left-sided tumors. This finding corresponded to left-sided predominant CMS2 tumors having better survival after relapse. In other words, systemic chemotherapy brought more therapeutic benefits for left-sided or CMS2 mCRCs. Nevertheless, tumor sidedness is still debated and rough for prognosis. More specific and detailed biomarkers and genetic profiles, instead of tumor sidedness, are necessary for analysis of prognostic impact.

According to our review of the relevant literature, this is one of the few studies to evaluate the prognostic values of EGFR expression, *KRAS* mutations, and tumor sidedness in patients with previously treated mCRC receiving salvage regorafenib plus FOLFIRI with dose-escalated irinotecan. The present study had some limitations. First, this was a retrospective, observational study with a relatively small sample size. We intend to conduct a prospective, randomized large-scale study to confirm the present study findings. Furthermore, extended *RAS* mutations, gene expression profiling for classification into CMS groups, and other potential regorafenib-related biomarkers should be included in future analyses.

Conclusions

The administration of regorafenib and concomitant reintroduction of FOLFIRI with dose-escalated irinotecan according to *UGT1A1* genotyping are clinically feasible and result in tolerable toxicities and favorable oncological outcomes in previously heavily treated mCRC. Positive EGFR expression has a negative effect on response to treatment as well as PFS and OS. *KRAS* mutations are associated with a poor OS and lower incidence of grade ≥ 3 AEs, which may serve as a clinical predictor for survival. Tumor sidedness is an independent prognostic factor for both PFS and OS. However, these findings must be validated by a further large-scaled randomized trial to address the

roles of EGFR expression, *KRAS* mutation status, and tumor sidedness in the relevant regorafenib combination therapy.

CRedit authorship contribution statement

Cheng-Jen Ma: Investigation, Formal analysis, Writing - original draft.

Ching-Wen Huang: Investigation.

Tsung-Kun Chang: Formal analysis. **Hsiang-Lin Tsai:** Formal analysis.

Wei-Chih Su: Investigation. **Yung-Sung Yeh:** Investigation.

Po-Jung Chen: Data curation. **Jaw-Yuan Wang:** Conceptualization, Methodology, Project administration.

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Conflict of Interests

The authors declare no conflict of interest.

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