Int Surg 2018;**103:**27–35
DOI: 10.9738/INTSURG-D-17-00050.1

Analysis of Mutational Spectra in Metastatic Colorectal Carcinoma: *KRAS* as an Indicator of Oxaliplatin-Based Chemotherapy

Chu-Cheng Chang[1,2], Jen-Kou Lin[1,2], Tzu-Chen Lin[1], Wei-Shone Chen[1,2], Jeng-Kai Jiang[1,2], Shung-Haur Yang[1,2], Huann-Sheng Wang[1,2], Yuan-Tzu Lan[1,2], Chun-Chi Lin[1,2], Shih-Ching Chang

[1]*Division of Colon and Rectal Surgery, Department of Surgery, Taipei Veterans General Hospital, Taipei, Taiwan*

[2]*National Yang-Ming University, Taipei, Taiwan*

Objective: Mutation spectra in colorectal cancer with metastasis and its response to chemotherapy.

Summary of Background Data: No molecular markers are available for selecting the optimal chemotherapeutic regimen (irinotecan or oxaliplatin) for metastatic colorectal cancer (mCRC).

Methods: We enrolled 161 mCRC patients who underwent surgery for their primary tumors at Taipei Veterans General Hospital from 2004 to 2010. The prevalence of gene mutations was measured and correlated with responses to different cytotoxic agents.

Results: We detected 1,836 mutations in 12 genes. *KRAS* mutants affected 44.3% of the tumors. The rate of good response was insignificantly higher for patients with *KRAS* mutant tumors who received oxaliplatin-based chemotherapy compared with patients with *KRAS* wild-type tumors (65.6% versus 47.0%; $P = 0.15$). For patients who received irinotecan-based chemotherapy, the rate of good response was similar in patients with wild-type (55.0%; n = 11) and those with *KRAS* mutant tumors (54.5%; n = 12; $P = 1$). In patients with *KRAS* mutant tumors treated with an oxaliplatin-based regimen, the overall survival was 38.5 months (95% CI: 26.6–50.5 months), which was insignificantly better than that for patients treated with an irinotecan-based regimen (30.4 months; 95% CI: 15.8–45.1 months; $P = 0.206$).

Corresponding author: Shih-Ching Chang, MD, PhD, Taipei-Veterans General Hospital, Department of Surgery, Division of Colorectal Surgery, No.201, Section 2, Shipai Road, Taipei, TW 11212.
Tel: (O) +886 2 28757544 110; (H) +886 2 28755855; Fax: +886 2 28757639; E-mail: changsc@vghtpe.gov.tw

Conclusions: Our data could not come to the conclusion that patient with *KRAS* mutation mCRC may have better response with oxaliplatin-based first-line chemotherapy. Further study is needed to confirm the relationship between gene mutation and chemotherapy response.

Key words: KRAS – mCRC – Oxaliplatin – Irinotecan

Colorectal carcinoma (CRC) is the most common cancer in Taiwan.[1] Half of the patients with CRC eventually develop distant metastasis, resulting in poor outcomes. With the introduction of cytotoxic and biologic agents to treat metastatic colorectal cancer (mCRC), the median overall survival (OS) increased from 12 to 30 months, and nearly up to 70% of patients could have a chance to receive at least 2 lines of treatment.[2–6] In addition to tumor aggressiveness, therapeutic responses impact patient outcomes significantly. In particular, rapid and deep tumor shrinkage could convert unresectable metastatic lesions into resectable lesions, thus prolonging patient life and improving quality of life.[7,8] Increased molecular knowledge and randomized clinical trial results have identified the association between mutations in *RAS* genes, specifically *KRAS* and *NRAS*, and anti-EGFR monoclonal antibody (mAb) effectiveness. This has led to selected use of this class of drugs in patients with *RAS*-wild-type CRC.[9]

On the other hand, no ideal molecular marker has been identified to aid in selection of cytotoxic agents. Because irinotecan and oxaliplatin are regarded as equally effective agents, the use of particular chemotherapeutic combinations often depends on patients' comorbidities as well as institutional or personal preferences.[10–13]

Recently, some *in vitro* studies showed that manipulation of mutant *KRAS* could alter expression of ERCC1 and affect sensitivity to oxaliplatin,[14,15] but this was not reproduced in clinical analysis. Previously, we established a high throughput MassARRAY platform (Sequenom, San Diego, California) that included 12 genes and 138 hotspots, and we detected 1,836 mutations in those 12 genes in 997 (79.8%) nonmetastatic CRCs.[16] In this study, we analyzed clinical data from patients with mCRC who received at least 6 administrations of first-line oxaliplatin-based or irinotecan-based chemotherapy. We determined carcinoembryonic antigen (CEA) ratios and overall survival (OS) as indicators for treatment responses. Here, we present the relationships between first-line chemotherapy regimens and tumor response as well as OS.

Materials and Methods

Clinical data

We enrolled 161 patients with mCRC who underwent surgery for their primary tumors at Taipei Veterans General Hospital from 2004 to 2010. The exclusion criteria were preoperative radiochemotherapy, emergency operations, or death within 30 days after surgery. Clinical information that was prospectively obtained and stored in a database included age, sex, personal and family medical history, location, tumor-node-metastasis (TNM) stage, differentiation, pathologic prognostic features and follow-up conditions. Chemotherapy regimens (FOLFOX or FOLFIRI) were chosen by clinical physicians. Following surgery, patients were monitored quarterly for the first 2 years and semi-annually thereafter. The follow-up protocol included physical examination, digital rectal examination, CEA analysis, chest X-ray, abdominal sonogram, and computed tomography (CT). If needed, proton emission tomography (PET) or magnetic resonance imaging (MRI) was arranged.

Tumor tissues

Before sample collection, written informed consent for tissue collection was obtained from all patients. Samples were meticulously dissected and collected from different quadrants of the tumors. Samples were immediately frozen in liquid nitrogen and stored in the Taipei Veterans General Hospital Biobank. Sections of cancerous tissue and corresponding normal tissue were reviewed by a senior gastrointestinal pathologist.

DNA isolation and quantification

After approval from the Institutional Review Board of the Taipei Veterans General Hospital, (2013-04-042B), samples for this study were obtained from the Biobank. DNA from tissue specimens was extracted

using the QIAamp DNA Tissue Kit (Qiagen, Valencia, California), according to the manufacturer's recommendations. DNA quality and quantity were confirmed using the Nanodrop 1000 Spectrophotometer (Scientific, Waltham, Massachusettes).

MassARRAY-based mutation characterization

The MassDetect CRC panel (v1.0), which enables the identification of 139 mutations in 12 genes (Supplementary Table 1), was designed as previously described.[16] Polymerase chain reaction (PCR) and extension primers for the mutations were designed using MassARRAY Assay Design 3.1 software (Sequenom, San Diego, California). The mutation alleles were manually designed by extension in either the forward or reverse direction to have lower masses than the reference alleles. After analyzing the primer designs with BLAST, any necessary modifications were made to avoid pseudogene amplification. MassARRAY-based mutation detection methods are described in Supplementary Table 1. PCR products of multiplexed reactions were spotted onto SpectroCHIP II arrays, and DNA fragments were resolved by on the MassARRAY Analyzer 4 System (Sequenom). Each spectrum was then analyzed using Typer 4.0 software (Sequenom) to identify mutations. Putative mutations were further filtered by manual review.

Microsatellite instability (MSI) analysis

Five reference microsatellite markers were used according to international criteria for determination of MSI: *D5S345*, *D2S123*, *BAT25*, *BAT26*, and *D17S250*. Primer sequences were obtained from GenBank (www.gdb.org). MSI detection was performed as previously described.[17,18] Briefly, DNA was amplified using fluorescent PCR. PCR products were denatured and analyzed by electrophoresis on 5% denaturing polyacrylamide gels, and results were analyzed using GeneScan Analysis software (Applied Biosystems, Carlsbad, California). Tumor samples that exhibited allele peaks different from the corresponding normal sample(s) were classified as having MSI for that particular marker. Samples with $\geq$2 MSI markers were defined as having MSI and those with 0–1 MSI markers were classified as having MSS.

CEA measurement and CEA ratio assessment

CEA levels were measured before chemotherapy and every 3 months after initial chemotherapy. The CEA ratio was defined as the CEA level 3 months after chemotherapy divided by the pretreatment CEA level (post-CEA/pre-CEA).

The serum CEA level was measured in the Department of Nuclear Medicine at the Taipei Veteran General Hospital. Thirty-nine patients whose CEA levels were within normal limits (<5 μg/L) throughout the treatment were excluded from the study. Thus, a total of 122 patients were enrolled in this study.

Similar to the Response Evaluation Criteria In Solid Tumors (RECIST) criteria, the tumor response to chemotherapy was defined according to the CEA ratio before analysis: complete response, having CEA level return to normal value; partial response, having CEA ratio $<$ 50% but CEA level above the normal value; stable disease, having 50% CEA ratio $<$ 100%; and progressive disease, having CEA ratio $>$ 100%.

Response assessment via imaging

CT or MRI were collected before chemotherapy and 3 months after treatment. Image changes were evaluated according to RECIST criteria. The RECIST criteria have limitations. Patients with disseminated metastasis or immeasurable metastasis could not be evaluated. Patients that were unable to be evaluated using RECIST criteria were excluded. Therefore, 77 patients were excluded. The imaging results for 85 patients were evaluable and considered in this study.

Statistical analysis

OS, which was defined as the date from surgery until death, was the statistical endpoint for analyses in this study. Patients not known to have died were censored at the date of last follow-up. Kaplan–Meier survival curves were plotted and compared using log-rank tests. The impact of chemotherapeutic regimens, clinicopathologic features, and genetic mutations on OS were assessed using Cox regression univariate and multivariate analyses. The chi-squared test and 2-tailed Fisher's exact test were used to compare genotype frequency according to clinicopathologic features. Numerical values were compared using Student's *t*-test. Data were expressed as mean ± standard deviation. Statistical significance was defined as $P < 0.05$. Statistical analyses were performed using SPSS for Windows (version 16.0).

Table 1 Demographic distribution of mCRC patient subtyped by first-time chemotherapy

Characteristics	Overall		Irinotecan-based		Oxaliplatin-based		*P* value
	(N = 122)	%	(N = 47)	%	(N = 75)	%	
Gender							
Male	77	63.1	34	72.3	43	57.3	0.123
Female	45	36.9	13	27.7	32	42.7	
Age, years							
Mean	66.2		65.6		65.9		
Median	67.6		65.9		66.8		
Range	35∼92		35∼89		37∼92		
Location							0.845
Colon	81	66.3	32	68.1	49	65.3	
Right	39	32	14	29.8	25	33.3	
Left	42	34.4	18	38.3	24	32	
Rectum	41	33.6	15	31.9	26	34.7	
Metastatic site							
Lung	22	18.2	1	2.2	21	28	0.001
Liver	88	72.1	36	74.5	53	70.7	0.684
Others	23	18.9	3	6.4	20	26.7	0.008
Pathologic features							
Poorly differentiation	10		6	12.8	4	5.3	0.181
LVI	52		17	36.2	35	46.7	0.267
Mucinous histology	9		6	12.8	3	4	0.086
Survival, months							0.635
Mean	30		35.9		29.5		
Median	21.7		24.3		23.7		
Range	1.53∼133.4		2.2∼133.3		1.5∼100.27		
CEA							0.922
Mean	322.4		313.7		329.8		
Median	46.8		41.9		52.4		

Results

There were 77 men (63.1%) and 45 women (36.9%) included in this study. The mean age at tumor resection was 66.2 ± 12.4 years (range, 35–92 years; median, 67.6 years). Patients were diagnosed with 39 proximal colon cancer (32.0%), 42 distal colon cancer (34.4%), and 41 rectal cancer (33.6%). The patients were also diagnosed with metastatic lesions in the liver (N = 88; 72.1 %), lungs (N = 22; 18.2 %), and other sites (N = 23; 18.9%) (Table 1).

Seventy-five patients were administered a median 8.6 cycles of FOLFOX as first-line treatment (range, 1–19 cycles). Forty-five patients received a median 9.4 cycles of FOLFIRI as first-line treatment (range, 1–32 cycles). The OS for all patients was 37.7 months (95% confidence interval [CI]: 29.5–45.8 months) in the FOLFOX group and 36.7 months (95% CI: 25.8–47.7 months) in the FOLFIRI group ($P = 0.635$) (Supplementary Fig. 1).

The most frequently mutation was loss of 18q (45.9%, N = 56), followed by *KRAS* (44.3%, N = 54), *TP53* (26.2%, N = 32), *APC* (26.2%, N = 32), *PIK3CA* (8.2%, N = 10), and *NRAS* (6.6%, N = 8) (Supplementary Fig. 2).Detailed mutation information for individual genes is described in Table 2.

Our previous study showed that CEA ratios (post-CEA/pre-CEA) and imaging changes according to RECIST criteria both correlated with OS and treatment responses.[19] In this study, we used CEA ratio (3 months posttreatment CEA/pretreatment CEA) as a parameter for evaluating chemotherapy response. Overall good responses were achieved in 53.5% (N = 23) of patients treated with FOLFIRI and 55.9% (N = 38) of patients treated with FOLFOX, when using CEA as an evaluation parameter ($P = 0.85$). Responses to oxaliplatin-based and irinotecan-based chemotherapy were not associated individual gene mutations, with the exception of a marginal effect for *KRAS* mutation (Supplementary Table 2). Detailed mutation information for individual genes response is described in Supplementary Table 3.

For patients who received oxaliplatin-based chemotherapy, the rate of good response was 47.0% (N = 16) for patients with wild-type *KRAS* tumors. This was insignificantly lower than that for patients with *KRAS* mutant tumors (65.6%, N = 21; $P = 0.15$). For patients who received irinotecan-based

Table 2 Distribution of overall patients

Variable	Irinotecan-based	%	Oxaliplatin-based	%	*P*
KRAS					0.708
Wild type	20	47.6	34	51.5	
Mutant	22	52.4	32	48.5	
BRAF					1
Wild type	41	97.6	63	95.5	
Mutant	1	2.4	3	4.5	
HRAS					1
Wild type	43	10	67	98.5	
Mutant	0	0	1	1.5	
NRAS					0.65
Wild type	38	88.4	64	94.1	
Mutant	5	11.6	3	4.4	
Loss of 18q					0.06
Wild type	16	37.2	39	57.4	
Mutant	27	62.8	29	42.6	
APC					0.23
Wild type	28	65.1	51	75	
Mutant	15	34.9	17	25	
SMAD4					1
Wild type	42	97.7	65	95.6	
Mutant	1	2.3	3	4.4	
TGFβ					1
Wild type	42	97.7	68	100	
Mutant	1	2.3	0	0	
TP53					0.31
Wild type	29	67.4	50	73.5	
Mutant	14	32.6	18	26.5	
PIK3CA					1
Wild type	40	93	61	89.7	
Mutant	3	7	7	10.3	
PTEN					0.39
Wild type	42	97.7	68	100	
Mutant	1	2.3	0	0	
FBXXW7					1
Wild type	40	93	67	98.5	
Mutant	3	7	1	1.5	
AKT1					1
Wild type	43	100	67	98.5	
Mutant	0	0	1	1.5	
MSI					0.341
Low	45	95.7	67	89.3	
High	2	4.3	8	10.7	

chemotherapy, the rates of good responses was similar for patients with wild-type *KRAS* tumors (55.0%; N = 11) and those with *KRAS* mutant tumors (54.5%; N = 12; $P = 1$) (Table 3).

The OS for patients with *KRAS* wild-type tumors who were treated with oxaliplatin-based first-line chemotherapy was 36.3 months (95% CI: 25.6–47.1 months), and OS was 42.3 months (95% CI: 25.6–59.0 months) for patients treated with an irinotecan-based regimen ($P = 0.702$).

In patients with *KRAS* mutant tumors who were treated with an oxaliplatin-based regimen, the overall survival was 38.5 months (95% CI: 26.6–50.5 months), which was insignificantly better than that for patients treated with an irinotecan-based regimen (30.4 months; 95% CI: 15.8–45.1 months; $P = 0.206$) (Fig. 1).

Table 3 Relationship between mutation, chemotherapy regimen, and response

	KRAS		
Parameter	Wild type	Mutant	*P*
CEA ratio			
FORFIRI			1
Good response	11 (55.0)	12 (54.5)	
Poor response	9 (45.0)	10 (45.5)	
FOLFOX			0.15
Good response	16 (47.0)	21 (65.6)	
Poor response	18 (53.0)	11 (34.4)	
RECIST			
FORFIRI			1
Good response	5 (38.5)	8 (42.1)	
Poor response	8 (61.5)	11 (57.9)	
FOLFOX			0.56
Good response	15 (55.6)	15 (65.2)	
Poor response	12 (44.4)	8 (34.8)	

KRAS as a predictor of response to oxaliplatin-based chemotherapy

The National Cancer Institute (NCI) database containing data from the 60 NCI60 cell lines was used as the source of cytotoxicity data for oxaliplatin and irinotecan (SN-38). The GI_{50}, which is the concentration required to inhibit growth by 50%, was used as a cytotoxicity parameter. Of these 60 cell lines, 53 had *KRAS* mutations status data, which was, identified from the COSMIC (Catalogue of Somatic Mutations in Cancer) database. As shown in Fig. 2, cell lines with *KRAS* mutations were more sensitive to oxaliplatin than wild-type *KRAS* cells. The GI_{50} values for mutant and wild-type *KRAS* cell lines were $10^{-6.21}$ and $10^{-5.66}$, respectively. This revealed that wild-type *KRAS* cells required 10 times more drug to inhibit growth than mutant *KRAS* cells. In contrast, SN-38 had a similar median GI_{50} in cell lines with/without *KRAS* mutation ($10^{-4.16}$ *versus* $10^{-4.05}$).

Discussion

Our study implies that using first-line oxaliplatin-based chemotherapy might result in better survival benefits for patients with *KRAS* mutant mCRC. Although not statistically significant, patients with *KRAS* mutant tumors who were treated with

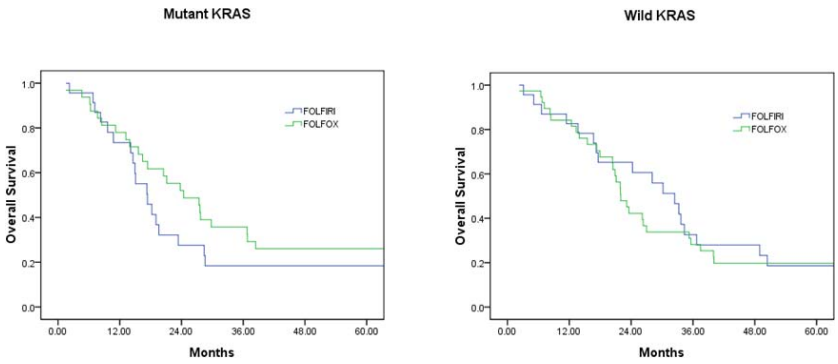

Fig. 1 Overall survival stratified by *KRAS* status. (A) Patients with mutant *KRAS* tumor who were treated with different chemotherapy regimens (P = 0.702). (B) Patients with wild-type *KRAS* tumors who were treated with different chemotherapy regimens (P = 0.206).

FOLFOX had a better response rate than patients treated with FOLFIRI (65.6% versus 54.5%, P = 0.15). This better response rate might lead to a higher chance of surgical resectability.[8,10]

The mutational profiling in our study revealed that loss of 18q (45.9%, N = 56), followed by *KRAS* (44.3%, N = 54), *P53* (26.2%, N = 32), *APC* (26.2%, N = 32), *PIK3CA* (8.2%, N = 10), and *NRAS* mutations (6.6%, N = 8) were most common among our patients with mCRC. The previous study by Russo *et al*, who reviewed 222 American patients with mCRC(20), showed a prevalence of patients with *KRAS* mutations (36%), followed by *TP53* mutations (21%), *APC* mutations (8%), *NRAS* mutations (4%), *BRAF* mutations (10%), and *PIK3CA* mutations (13%). The *APC* mutations were much more frequent in our database.

The OS rates for patients with mutant *KRAS* tumors who underwent oxaliplatin-based or irinotecan-based regimens were 38.5 months and 30.4 months, respectively, in our study. In the PRIME study, the OS for patients with *KRAS* mutant tumors who were treated with FOLFOX4 alone was 19.3 months.[5] In the CRYSTAL study, the OS for patients with *KRAS* mutant tumors who were treated with FOLFIRI was 17.7 months.[21] Overall survival was higher in our study. This was mainly because the patient in our study underwent surgical resections for their primary tumors. The response rate of patients with *KRAS* mutant tumors in the CRYSTAL study was 40.2% (FOLFIRI), and it was 40% in the PRIME study (FOLFOX4). The response rates were also higher in our study (65.6% for oxaliplatin-based and 55% for irinotecan-based). Our data was collected from 2004 to 2010, when targeted agent therapy had not yet been reimbursed by the National Health Insurance System in Taiwan.

In recent years, CRC molecular and cytogenetic characteristics have allowed to increasingly understand CRC. Several biomarkers have been identified and confirmed to predict progression and survival.[16–18,22] Our study tried to compare different biomarkers to oxaliplatin-based or irinotecan-based chemotherapy alone in response rate for patients with mCRC. We revealed an approximately 65% response rate in patients with *KRAS* mutant tumors who were treated with FOLFOX compared to the 42.1–54.5% for patients with *KRAS* mutant tumors who were treated with irinotecan-based chemotherapy. Thus FOLFOX elicited a 35.3% better response rate. Although the OS for patients with *KRAS* mutant tumors who were treated with first-line oxaliplatin or irinotecan based chemotherapy was not significantly different (P = 0.20), oxaliplatin-based first-line chemotherapy tended to result in a more favorable outcome.

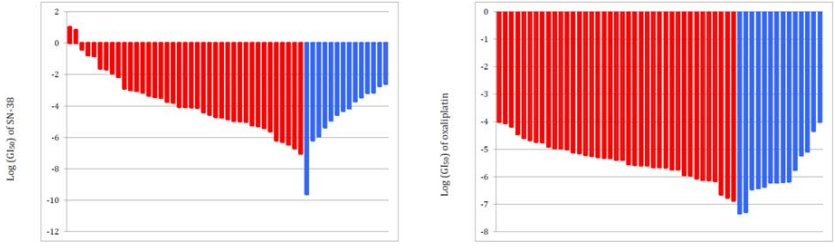

Fig. 2 (A) The log (GI_{50}) of SN-38 in 53 NCI60 panel cell lines. The median log(GI_{50}) is -4.05 for 39 wild-type *KRAS* cell lines (red), which was similar to that for the 14 mutant *KRAS* cell lines (-4.16; blue). (B) The log (GI_{50}) of oxaliplatin in 53 NCI60 panel cell lines. The median log(GI_{50}) is -5.33 for 39 wild-type *KRAS* cell lines (red), which was higher than that for the 14 mutant *KRAS* cell lines (-6.21; blue).

According to Huang *et al*, CEA ratio and imaging change according to RECIST criteria both correlated with overall survival.[19] In addition to using CEA ratio as a parameter, we have also used RECIST criteria to evaluate treatment response rates. The overall response rate was 39.4% (N = 13) for patients treated with FOLFIRI and 57.7% (N = 30) for patients treated with FOLFOX ($P = 0.12$) (Supplementary Table 2). When comparing the patients with tumors that have wild-type or mutant *KRAS*, oxaliplatin-based regimens elicited a slightly better response rate than irinotecan-based regimens. In patient who were administered oxaliplatin-based chemotherapy, the rates of good responses were 55.6% (N = 15) for those with *KRAS* wild-type tumors and 65.2% (N = 15) for those with *KRAS* mutant tumors ($P = 0.56$). For patients who were administered irinotecan-based chemotherapy, the rate of good responses were 38.5% (N = 5) for those with *KRAS* wild-type tumors, which was similar to that for patients with *KRAS* mutant tumors (42.1%; N = 8; $P = 1$). This result also suggested more favorable outcomes for patients with *KRAS* mutant tumors who receive oxaliplatin-based first-line chemotherapy. We've also used RECIST criteria to assess other mutational factors. The rates of good response for patients with wild-type loss of 18q were 15.4% (N = 2) when treated with FOLFIRI and 46.7% (N = 14) when treated FOLFOX ($P = 0.032$). The response rates for patient with mutant loss of 18q treated with FOLFIRI or FOLFOX were 55% (N = 11) and 72.7% (N = 16) ($P = 0.089$), respectively. For patients with tumors expressing wild-type *APC*, the rates of good responses were 28.6% (N = 6) when treated FOLFIRI and 51.9% (N = 27) with FOLFOX ($P = 0.31$). In patients with tumors expressing mutant *APC*, the rate of good response were 46.7% (N = 7) when treated with FOLFIRI and 68.4% (N = 13) with FOLFOX ($P = 0.283$). For patients with tumors expressing wild-type *P53*, the rates of good responses were 33.3% (N = 9) when treated with FOLFIRI, and 61.9% (N = 27) with FOLFOX ($P = 0.693$). For patients with tumors expressing mutant *P53*, the rates of good responses were 44.4% (N = 4) when treated with FOLFIRI, and 68.4% (N = 13) with FOLFOX ($P = 0.283$) (Supplementary Table 4).

The superior results after oxaliplatin treatment in patients with *KRAS* mutant tumor are comparable to the result of Lin *et al*.[14,15] For mCRC patients treated with oxaliplatin-based chemotherapy, the median progression-free survival (PFS) was 8.5 months for patients with *KRAS* mutant tumors versus 5.8 months for patients with *KRAS* wild-type tumors ($P = 0.008$). However, for patients treated with irinotecan-based chemotherapy, the median PFS was 3.9 months for patients with *KRAS* mutant tumors versus 6.0 months for patients with *KRAS* wild-type tumors ($P = 0.23$).

Furthermore, according to an recent *in vitro* study by Lin *et al*,[15] *KRAS* mutation is a predictor of oxaliplatin sensitivity in colon cancer cells. In the *in vitro* study, *KRAS* mutant CRC cells were more sensitive to oxaliplatin than *KRAS* wild-type CRC cells. Overexpression of excision repair cross-complementation group 1 (ERCC1) is associated to resistance to platinum-based chemotherapy. Mutant *KRAS* cells might downregulate ERCC1 through hypermethylation of ERCC1 gene. In the *in vitro* study, using the apoptosis rate 48 hours after platinum-based treatment, the response rate was 22.5% for *KRAS* wild-type CRC wells versus 39.1% for *KRAS* mutant cells. The results implied that *KRAS* mutated CRC cells are more sensitive to oxaliplatin-based treatment. The role of ERCCI was discussed in a previous study. Chua *et al*[23] showed that ERCC1 is associated with worse progression-free survival in patients treated with FOLFOX. H Baba *et al*[18] also reported higher ERCC1 levels lead to resistance to oxaliplatin. Furthermore, *KRAS* mutated cell lines were more sensitive to oxaliplatin *in vitro*.[24]

The National Cancer Institute (NCI) database containing data from the NCI60 cell lines was used as the source of cytotoxicity data for oxaliplatin. The GI_{50}, which is the concentration required to inhibit growth by 50%, was used as a parameter for cytotoxicity. Of these 60 cell lines, there was information about the *KRAS* mutation status for 53 lines. The median log (GI_{50}) was -5.33 in 39 wild-type *KRAS* cell lines when treated with oxaliplatin. This result was higher than that for 14 mutant *KRAS* cell lines (-6.21). The cell line data revealed that a higher concentration of oxaliplatin was needed to inhibit tumor growth in wild-type *KRAS* cells. In the contrast, when cell lines were treated with SN-38, the result was similar for both groups (-4.05 in *KRAS* wild-type cell lines versus -4.16 in *KRAS* mutant cell lines). This result further strengthened our results in this study.

There are limitations to our study. First, it was a retrospective study with a relatively small sample size. Limited power might result in statistical insignificance in our study. A larger prospective study is needed to confirm the result. However, it is hard to design this kind of study. Target therapy has been the protocol for first-line chemotherapy for

patients with mCRC. However, *in vitro* studies might help confirm the results of oxaliplatin resistance using different molecular markers. Second, we lack data on progression-free survival, which is an indicator of treatment response.

Our data could not come to the conclusion that patient with *KRAS* mutation mCRC may have better response with oxaliplatin-based first-line chemotherapy. Further study is needed to confirm the relationship between gene mutation and chemotherapy response.

Acknowledgments

The authors report no conflicts of interest. There is no source or support for this paper.

References

1. Department of Health tEY. Cancer registry annual report. Taiwan, R.O.C.: The Department of Health, the Executive Yuan; 2011.
2. Falcone A, Cremolini C, Masi G, Lonardi S, Zagonel V, Salvatore L *et al*. FOLFOXIRI/bevacizumab (bev) versus FOLFIRI/bev as first-line treatment in unresectable metastatic colorectal cancer (mCRC) patients (pts): Results of the phase III TRIBE trial by GONO group. *J Clin Oncol* 2013; **31**(15_suppl):3505–3505
3. Stintzing S, Fischer von Weikersthal L, Decker T, Vehling-Kaiser U, Jager E, Heintges T *et al*. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer-subgroup analysis of patients with KRAS: mutated tumours in the randomised German AIO study KRK-0306. *Ann Oncol* 2012;**23**(7):1693–1699
4. Van Cutsem E, Kohne CH, Lang I, Folprecht G, Nowacki MP, Cascinu S *et al*. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol* 2011; **29**(15):2011–2019
5. Douillard J-Y, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M *et al*. Randomized, Phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol* 2010;**28**(31):4697–4705
6. Kopetz S, Chang GJ, Overman MJ, Eng C, Sargent DJ, Larson DW *et al*. Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy. *J Clin Oncol* 2009;**27**(22):3677–3683
7. Cremolini C, Schirripa M, Antoniotti C, Moretto R, Salvatore L, Masi G *et al*. First-line chemotherapy for mCRC-a review and evidence-based algorithm. *Nat Rev Clin Oncol* 2015;**12**(10): 607–619
8. Stintzing S, Modest DP, von Weikersthal LF, Decker T, Kiani A, Vehling-Kaiser U *et al*. Independent radiological evaluation of objective response, early tumor shrinkage, and depth of response in FIRE-3 (AIO KRK-0306) in the final RAS evaluable population. *Ann Oncol* 2014;**25**:1
9. De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G *et al*. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010;**11**(8):753–762
10. Van Cutsem E, Cervantes A, Nordlinger B, Arnold D. Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2014; **25 Suppl 3**:iii1–9
11. Tournigand C, Andre T, Achille E, Lledo G, Flesh M, Mery-Mignard D *et al*. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 2004;**22**(2):229–237
12. Colucci G, Gebbia V, Paoletti G, Giuliani F, Caruso M, Gebbia N *et al*. Phase III randomized trial of FOLFIRI versus FOLFOX4 in the treatment of advanced colorectal cancer: a multicenter study of the Gruppo Oncologico Dell'Italia Meridionale. *J Clin Oncol* 2005;**23**(22):4866–4875
13. Pox C, Aretz S, Bischoff SC, Graeven U, Hass M, Heuner P *et al*. S3-Leitlinie Kolorektales Karzinom Version 1.0 – Juni 2013 AWMF-Registernummer: 021/007OL. *Z Gastroenterol* 2013; **51**(08):753–854
14. Lin YL, Liau JY, Yu SC, Tseng LH, Lin LI, Liang JT *et al*. Oxaliplatin-based Chemotherapy Might Provide Longer Progression-Free Survival in KRAS Mutant Metastatic Colorectal Cancer. *Transl Oncol* 2013;**6**(3):363–369
15. Lin YL, Liau JY, Yu SC, Ou DL, Lin LI, Tseng LH *et al*. KRAS mutation is a predictor of oxaliplatin sensitivity in colon cancer cells. *PLoS One* 2012;**7**(11):e50701
16. Mouradov D, Domingo E, Gibbs P, Jorissen RN, Li S, Soo PY *et al*. Survival in stage II/III colorectal cancer is independently predicted by chromosomal and microsatellite instability, but not by specific driver mutations. *Am J Gastroenterol* 2013; **108**(11):1785–1793
17. Sarli L, Bottarelli L, Bader G, Iusco D, Pizzi S, Costi R *et al*. Association between recurrence of sporadic colorectal cancer, high level of microsatellite instability, and loss of heterozygosity at chromosome 18q. *Dis Colon Rectum* 2004;**47**(9):1467–1482
18. Baba H, Watanabe M, Okabe H, Miyamoto Y, Sakamoto Y, Baba Y *et al*. Upregulation of ERCC1 and DPD expressions after oxaliplatin-based first-line chemotherapy for metastatic colorectal cancer. *Br J Cancer* 2012;**107**(12):1950–1955

19. Huang SC, Lin JK, Lin TC, Chen WS, Yang SH, Wang HS *et al*. Concordance of carcinoembryonic antigen ratio and response evaluation criteria in solid tumors as prognostic surrogate indicators of metastatic colorectal cancer patients treated with chemotherapy. *Ann Surg Oncol* 2015;**22(**7):2262–2268
20. Russo AL, Borger DR, Szymonifka J, Ryan DP, Wo JY, Blaszkowsky LS *et al*. Mutational analysis and clinical correlation of metastatic colorectal cancer. *Cancer* 2014; **120**(10):1482–1490
21. Van Cutsem E, Kohne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A *et al*. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009; **360**(14):1408–1417
22. Bacolod MD, Barany F. Molecular profiling of colon tumors: the search for clinically relevant biomarkers of progression, prognosis, therapeutics, and predisposition. *Ann Surg Oncol* 2011;**18**(13):3694–3700
23. Chua W, Goldstein D, Lee CK, Dhillon H, Michael M, Mitchell P *et al*. Molecular markers of response and toxicity to FOLFOX chemotherapy in metastatic colorectal cancer. *Br J Cancer* 2009; **101**(6):998–1004
24. Orlandi A, Di Salvatore M, Bagala C, Basso M, Strippoli A, Plastino F *et al*. ERCC1 Induction after Oxaliplatin Exposure May Depend on KRAS Mutational Status in Colorectal Cancer Cell Line: In Vitro Veritas. *J Cancer* 2015;**6**(1):70–81