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Role of *CCL5* and *CCR5* gene polymorphisms in EGFR signaling blockade in Metastatic Colorectal Cancer: Analysis of the FIRE-3 trial

Mitsukuni Suenaga¹, Sebastian Stintzing², Shu Cao³, Wu Zhang¹, Dongyun Yang³, Yan Ning¹, Satoshi Okazaki¹, Martin D. Berger¹, Yuji Miyamoto¹, Marta Schirripa¹, Shivani Soni¹, Afsaneh Barzi¹, Volker Heinemann², and Heinz-Josef Lenz¹

¹Division of Medical Oncology Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, 1441 Eastlake Avenue, Los Angeles, CA 90033, USA

²Department of Medicine III, University Hospital, LMU Munich, Marchioninistrasse 15, 81377 Munich, Germany

³Department of Preventive Medicine, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, 1441 Eastlake Avenue, Los Angeles, CA 90033, USA

Abstract

Background: Epidermal growth factor receptor signaling blockade increases *CCL5* expression that regulate either the anti-tumor immune response or tumor progression. We investigated the potential role of *CCL5/CCR5* axis in cetuximab-based treatment in metastatic colorectal cancer (mCRC) patients.

Patients and methods: Genomic DNA was extracted from 491 samples of two different cohorts with *KRAS* wild-type mCRC from the FIRE-3 trial: an evaluation cohort of 244 patients receiving cetuximab plus FOLFIRI; and a control cohort of 247 patients receiving bevacizumab plus FOLFIRI. Single nucleotide polymorphisms (SNPs) of *CCL5* and *CCR5* genes were analyzed by PCR-based direct sequencing.

Results: Patients in the evaluation cohort with any *CCL5* rs2280789 G allele had shorter overall survival (OS) compared with those with the A/A variant (HR 1.56, $P=0.024$). Patients carrying any *CCR5* rs1799988 T allele had a trend toward lower response rate than those with the C/C variant (68 vs. 81%, $P=0.078$). In the analysis based on primary tumor location (left-sided [L]: right-sided [R]), remarkable differences in outcomes were observed between patients with L-*CCR5* SNPs C/C variant (L-C/C), L-any T, R-T/T and R-any C as follows: median OS, 38.5, 30.6, 27.1, 15.8

Corresponding author: Mitsukuni Suenaga, M.D., Ph.D., Division of Medical Oncology, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, 1441 Eastlake Ave, Suite 3456, Los Angeles, CA, 90033, United States., Tel: +1-(323)-865-3967, Fax: +1-(323)-865-0061, m.suenaga1972@gmail.com.

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Disclosure

The authors have no conflicts of interest to declare in this work.

months, $P<0.001$; response rate, 91, 66, 92, 48%, $P<0.001$. Median OS for *CCL5* SNPs including L-A/A, L-any G, R-A/A and R-any G groups were 38.3, 21.7, 21.9, 18.3 months, $P<0.001$. The findings were not significant in the control cohort.

Conclusion: Genetic variants of *CCL5* and *CCR5* SNPs may predict outcomes in mCRC patients receiving cetuximab-based treatment depending on tumor location.

Keywords

CCL5; *CCR5*; cetuximab; metastatic colorectal cancer; primary tumor location

Introduction

Previously reported randomized trials have clearly proven the clinical impact of first-line chemotherapy plus either anti-epidermal growth factor receptor (EGFR) or anti-vascular endothelial growth factor (VEGF) antibodies for metastatic colorectal cancer (mCRC) [1,2]. According to the subgroup analyses regarding the tumor molecular subtype and primary tumor location, recent guidelines suggest that anti-EGFR antibodies cetuximab and panitumumab are preferred in *RAS* wild-type tumors located in the left side of the colon or rectum. Meanwhile, the anti-VEGF antibody bevacizumab is recommended for patients regardless of either *RAS* status or primary tumor location [3,4]. However, how to predict efficacy of the biologic agents has not been sufficiently clarified to date.

EGFR signaling blockade increases C-C motif chemokine ligand-5 (*CCL5*) expression, which attracts tumor-infiltrating leukocytes that regulate either the host-derived anti-tumor immunity or tumor progression [5–7]. A high degree of T cell infiltration in cancer tissue is known to be associated with a favorable prognosis in colorectal cancer. T helper type 1 (Th1) expresses C-C motif chemokine receptor-5 (*CCR5*) and C-X-C motif chemokine receptor-3 (*CXCR3*), whereas Th2 expresses *CCR4*; and *CCR5*- and *CXCR3*-expressing T cells are recruited in to the invasive margin as anti-tumor immune responses. *CCL5* is expressed and localized within CD8+ T cells, while a *CXCR3* ligand *CXCL10/IP-10* is localized in tumor cells and macrophages within the invasive margin [8]. Stimulation of EGFR down-regulates *CCL2*, *CCL5* and *IP-10*, while it increases *CXCL8/IL-8* expression in normal cells. Conversely, EGFR signaling blockade produces opposite effects, with increased *CCL2*, *CCL5*, and *IP-10*, and reduced *IL-8* expression. Inhibition of EGFR signaling might exert antitumor activity by favoring the recruitment of inflammatory cells and a more pronounced anti-tumor immune response, along with down-regulation of *IL-8*, which is an important growth factor for malignant epithelial cells [6,7]. Expression of *IL-8/CXCR2* in the tumor microenvironment has been shown to play a critical role in progression and metastases with increased tumor angiogenesis in colon cancer [9]. In addition, the *CCL5/CCR5* axis participates in VEGF-A production by inducing endothelial progenitor cell migration [10] (Fig. A. 1). Meanwhile, a novel immune escape in CRC via *CCL5/CCR5* axis that enhances tumor's ability to beat antitumor CD8+ T cells by forming infiltration of T-regulatory cells [11]. Nevertheless, it still remains unclear how EGFR signaling blockade regulate either the host-derived anti-tumor immunity or tumor progression via *CCL5* activation in colorectal cancer patients.

We therefore tested whether genetic polymorphisms in *CCL5* and *CCR5* genes could predict efficacy of cetuximab or bevacizumab plus FOLFIRI in first-line treatment in metastatic colorectal cancer patients from the FIRE-3 trial (Trial Registration: NCT00433927).

Materials and methods

Study design and patients

Two different cohorts with *KRAS* exon 2 wild-type mCRC from the randomized phase III FIRE-3 trial [1] were investigated in this study: an evaluation cohort of 244 patients receiving cetuximab plus FOLFIRI; and a control cohort of 247 patients receiving bevacizumab plus FOLFIRI. All patients fulfilled the eligible criteria; no history of prior treatment for mCRC, measurable or evaluable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.0, and signed informed consent. Cetuximab (400 mg/m² followed by 250 mg/m² thereafter) and bevacizumab (5 mg/kg) were administered weekly and every 2 weeks, respectively. FOLFIRI (irinotecan 180 mg/m², 5-FU bolus 400 mg/m², 5-FU infusion 2400 mg/m², leucovorin 200 mg/m²) was administered every 2 weeks. Treatment was continued until any of the following occurred: disease progression, unmanageable toxicity, or patient refusal. The current study was approved by the Institutional Review Board of each participating site, and the molecular analyses were conducted at the University of Southern California/Norris Comprehensive Cancer Center in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. We were fully compliant with the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) guidelines.

Selection of candidate single-nucleotide polymorphisms (SNPs)

The two candidate SNPs of genes *CCL5* and *CCR5* were selected using any of the following criteria: i) SNP with biological significance according to a published literature review, ii) tagging SNPs selected by the HapMap genotype data with r^2 threshold = 0.8: <http://snpinfo.niehs.nih.gov/snpinfo/snptag.php>, or iii) minor allele frequency > 10% in Caucasians (in the Ensembl Genome Browser: <http://uswest.ensembl.org/index.html>). Functional significance was predicted based on the functional single-nucleotide polymorphism (F-SNP) database: <http://compbio.cs.queensu.ca/F-SNP/> (Table A. 1).

DNA extraction and genotyping

Genomic DNA was extracted from peripheral whole blood in patients of all cohorts using the QIAmp Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol (www.qiagen.com). The candidate SNPs were tested using PCR-based direct DNA sequence analysis by ABI 3100A Capillary Genetic Analyzer and Sequencing Scanner v1.0 (Applied Biosystems). Both forward and reverse primers used for amplification of extracted DNA are listed in Table A. 1. For quality control purposes, a randomly selected 10% of the samples was analyzed by direct DNA sequencing for each SNP, and the genotype concordance rate was of 99% or more. The investigators analyzing SNPs were blinded to the clinical data.

Statistical analysis

The primary endpoint of the current study was progression-free survival (PFS), and secondary endpoints were overall survival (OS) and objective response rate (ORR). PFS was defined as the interval between the date of randomization and the date of confirmed disease progression or death. OS was calculated from the date of randomization until the date of death from any cause. Data of patients without disease progression or death were censored at the date of last follow up. For patients who were lost to follow-up, data were censored at the date when the patient was last confirmed to be alive. The ORR was calculated from the number of patients who achieved complete response (CR) and partial response (PR) to treatment, according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0. Chi-square tests were used to examine the difference in baseline patient characteristics between two cohorts. Fisher's exact test was applied to examine the associations between SNPs and response. Association between SNPs and PFS and OS were estimated by the Kaplan-Meier method and were compared using the log-rank test, with predictive or prognostic clinical factors and candidate SNPs that was identified by univariate analysis using codominant, dominant, or recessive genetic model if appropriate. Multivariable analysis using the Cox proportional hazards model was then conducted to re-evaluate factors influencing PFS and OS. The baseline demographic and clinical characteristics that remained statistically significantly associated with PFS and OS in multivariable analyses were included in the final models. We further performed subgroup analyses by primary tumor site, then built the models combining tumor site and SNPs according to the results from subgroup analyses. With 244 patients (206 PFS events) in the evaluation cohort, we would have 80% power to detect a minimum hazard ratio (HR) of 1.49 to 1.65 for a SNP with minor allele frequency of 0.1 to 0.5 on PFS using a two-sided 0.05 level log-rank test. The power would be greater than 80% in the control cohort with 247 patients (203 PFS events) when applying the same test and assuming the same allele frequencies. Analysis in the RAS wild-type patients was also performed. All analyses were carried out with SAS 9.4 (SAS Institute, Cary, NC, USA). All tests were 2-sided at a significance level of 0.050.

Results

Baseline patients and tumor characteristics

The median follow-up time, median PFS and OS were 34.1 months, 9.8 months and 29.7 months in the evaluation cohort; and 39.4 months, 10.2 months and 24.8 months in the control cohort, respectively. The baseline characteristics of the evaluation and control are summarized in Table A. 2. The associations between baseline characteristics and clinical outcome are summarized in Table A. 3 and 4 for the evaluation and control cohorts, respectively. In detail, right-sided location, *RAS* and *BRAF* mutant were significantly associated with shorter PFS and OS in the evaluation cohort. In the control cohort, higher ECOG, right-sided location and *BRAF* mutant were significantly correlated with shorter PFS and OS. Frequencies of genetic variants of the SNPs satisfied the Hardy–Weinberg equilibrium ($P>0.01$) using the exact test in the Haploview software version 4.2.

Association of clinical outcome and SNPs tested in the evaluation cohort

In the evaluation cohort, patients with any *CCL5* rs2280789 G allele had shorter OS compared to those carrying the A/A variant in the univariate analysis (19.9 vs. 33.4 months, HR 1.56, 95% confidence interval (CI): 1.05–2.30, $P=0.024$); this effect was confirmed by the multivariable analysis (HR 1.57, $P=0.030$). Patients carrying any *CCR5* rs1799988 T allele had a lower response rate (68 vs. 81%, $P=0.078$) and significantly shorter PFS in the multivariable analysis than those with the C/C variant (9.4 vs. 10.6 months, HR 1.40, 95% CI: 1.01–1.93, $P=0.043$) (Table 1).

Subgroup analysis by primary tumor location showed that patients with any *CCL5* rs2280789 G allele had shorter PFS and OS compared to those with A/A variant in both right and left-sided subgroups; however, neither of these effects reached statistical significance. For *CCR5* rs1799988, the effects were in opposite direction between right and left-sided; the T/T variant was favorable in right-sided tumors, while T allele was unfavorable in left-sided tumors for tumor response, PFS and OS (Table 2). The phenomena were clearly identified in models combining SNPs and tumor location (Fig. 1). Statistically significant differences were shown among the groups consisting of primary tumor location and *CCL5* or *CCR5* SNPs (Model 1, Groups I–IV; Model 2, Groups I–IV). Similar effect was found in the *RAS* wild-type populations (Table 2). In model 1, any *CCL5* rs2280789 G allele was associated with poor tumor response, shorter PFS and OS compared with the A/A variant regardless of tumor location, suggesting similar allelic characteristics between tumor locations. In model 2, patients with right-sided tumor and any *CCR5* C allele (Group IV) had poor tumor response, shorter PFS and OS compared with those with a left-sided tumor and the *CCR5* C/C variant (Group I), suggesting an opposite allelic effects between tumor locations (Table 2).

We further developed a novel classification model composed of tumor location, and both *CCL5* and *CCR5* SNPs, which stratified patients into three categories: Group I, Group II and Group III (Fig. 2). Significant differences in tumor response, PFS and OS were observed among the three categories in both *KRAS* and *RAS* wild-type populations (Table 2, Fig. 3A–3C).

Association of clinical outcome and SNPs tested in the control cohort

In the control cohort, there was no significant association between SNPs and clinical outcomes in either univariate or multivariate analyses (Table 1). The novel classification model showed a significant association with OS for *KRAS* wild-type patients in only univariate analyses ($P=0.025$, adjusted $P=0.20$, Table A. 5). The interaction test for the classification groups and cohorts was then performed using the same multivariable Cox regression model for OS, which showed a significant association ($P<0.001$). Fig. 3D demonstrates K-M curves of the evaluation cohort along with the control cohort based on the classification. Accordingly, patient OS could be remarkably differentiated among the groups and cohorts, suggesting Group I in the evaluation cohort was likely to achieve the best OS, while Group III in the evaluation cohort tended to have poorer outcomes compared with the rest.

Discussion

Our data show the first evidence that SNPs of *CCL5* and *CCR5* genes are associated with clinical outcomes in first-line cetuximab plus FOLFIRI in patients with mCRC. In addition, the novel classification suggests that cetuximab-based treatment could be a possibility in right-sided mCRC, although the current guidelines do not recommend the use of anti-EGFR antibodies in a front-line setting in right-sided mCRC [3].

A potential role of CCL5/CCR5 in cancer progression has been demonstrated in various cancer types [8,12]. The CCL5/CCR5 axis is involved in the immune microenvironment and tumor progression through recruitment of specific immune cells by regulating either the host-derived anti-tumor immunity or tumor progression along with the concomitant chemokines [5,13,14]. A RNA expression analysis study of chemokines and chemokine receptors using RT-PCR in colorectal cancer demonstrated widely expressed CCL5 not only in cancer tissue but also in non-neoplastic mucosal tissues [8]. According to a clinicopathological study of colorectal liver metastases, T lymphocytes infiltrating into the invasive margin produced CCL5 delivered by tumor-infiltrating lymphocytes through CXCR3-mediated migration. By contrast, CCR5 was evident in all samples from CRC liver metastases though other CCL5 receptors, CCR1 and CCR3 were only localized on immune cells [15]. We thus expected that the CCL5/CCR5 signaling correlates with efficacy of the chemotherapeutic treatment for mCRC investigated in our study.

SNPs of *CCL5* or *CCR5* genes have been investigated in various cancers; however, the exact role of the alleles has not yet been clarified as pro-oncogenic action or response to chemotherapy in cancers, including colorectal cancer [16–23]. Considering genetic functionalities of SNPs, An et al. demonstrated that transcriptional regulation of CCL5 was primarily governed by *CCL5* rs2280789 in the promoter region, of which the G allele corresponded with a strong decrease in transcriptional activity of CCL5 [24]. To our knowledge, there have been no published reports on the impact of the *CCR5* rs1799988 on cancer susceptibility or its functional consequence on genes/proteins.

The most interesting findings in our study are that the clinical impact of *CCR5* SNPs differed depending on primary tumor location, whereas *CCL5* SNPs showed a similar trend regardless of tumor location. Furthermore, both the SNPs and primary tumor location had greater influence on OS than PFS. Considering not significant difference in PFS between cetuximab FOLFIRI arm and bevacizumab plus FOLFIRI arm in the FIRE-3 final *RAS* wild-type subgroup [1], the predictive value of those factors could not be neglected though its impact was smaller than prognostic value. The trend in OS and PFS was also confirmed in a pooled analysis consist of six randomized trials (CRYSTAL, FIRE-3, CALGB 80405, PRIME, PEAK and 20050181), comparing chemotherapy plus EGFR antibody with chemotherapy with or without bevacizumab [25]. As proposed in the study, it is interesting to note that a doublet plus EGFR antibody remains an option if treatment goal is tumor shrinkage; however, which still continues to confuse physician in treatment selection for *RAS* wild-type right-sided mCRC. In terms of tumor shrinkage, our novel classification could identify more clearly who benefit from chemotherapy plus EGFR antibody than tumor sidedness, albeit the trend in PFS and OS is similar between them. This suggests that the

distribution of genetic variant of the SNPs and primary tumor location were not completely matching each other, and our novel classification seems to cover the ambiguity of primary tumor location in predicting tumor response. A recent study reported the *in vivo* distribution of the Human Immunodeficiency Virus/Simian Immunodeficiency Virus co-receptors, CXCR4, CCR3, and CCR5 in human and rhesus monkey [26]. In the study, the expression levels of co-receptors were higher in the proximal than the distal part of the GI tract, i.e., colon than rectum, which might be potentially regulated by the degree of cellular maturation and activation, and the concentration of the related chemokines. Although the findings regarding the co-receptor expression distributions are extremely informative, they should be confirmed by further studies of cancer patients. We therefore speculate that such mediators regulate CCR5 expression according to tumor location in CRC leading to the opposite outcomes. Meanwhile, CCR5 is known to participate in the pathogenesis of inflammatory bowel disease (IBD) [27]; and a recent study reported a correlation of CCR5 expression and β -arrestin2 expression in intestinal mucosa with leukocyte infiltration in IBD [28]. β -arrestin2 is a cellular soluble protein that negatively regulates G protein-coupled receptors such as CCR5 to be desensitized or internalized. In addition, the consensus molecular subtypes (CMSs) of colorectal cancer have been introduced as the most robust classifier for CRC based on biology but not outcome [29], of which CMS1 is categorized as microsatellite instability (MSI) immune harboring immune infiltration and activation. CMS1 has been shown to localize in right-sided more frequently than in left-sided CRC, and correlate with a worse prognosis in mCRC. Taken together, such variation in immune environment between right- and left-sided tumors may account for the diverse manner of *CCR5* SNPs in the mechanism of action of cetuximab. Moreover, in a subgroup analysis of a randomized Phase III trial (CALGB/SWOG80405) comparing CET-based to BEV-based first-line chemotherapy, CMS1 was also frequently shown in right-sided tumors and revealed to be a potentially negative predictor of cetuximab [30]. We think the results could support the abovementioned our hypothesis. As next step in treatment for mCRC patients, immune checkpoint inhibitors targeting programmed death-1 (PD-1) and cytotoxic T-cell lymphocyte antigen-4 (CTLA-4) have demonstrated promising outcome, which focused on patients with DNA mismatch repair-deficient (dMMR)/MSI-high (MSI-H) mCRC [31,32]. Although crosstalk between EGFR signaling and PD-1 pathway still remains unclear, which urges us to develop possible combination of EGFR antibody and PD-1 and/or -CTLA-4 inhibitor regarding the poor prognosis in patients with dMMR/MSI-H mCRC. Recently, a phase Ib/II study reported the tolerability of combination of cetuximab and pembrolizumab in patients with *RAS* wild-type mCRC and no additional emergent toxicity was observed. The efficacy data in the ongoing phase II study is warranted [33].

The strengths of our study are as follows: the two cohorts derived from a randomized phase III trial (FIRE-3) comparing cetuximab-based with bevacizumab-based chemotherapy; and the consistent results between the *KRAS* wild-type and *RAS* wild-type populations in the evaluation cohort but not in the control cohort. As limitations of our study, we have to perform further preclinical and validation studies to explore the biology of *CCR5* SNPs, which differed by primary tumor location for clinical outcome in cetuximab-based treatment. In addition, correlation between expression of *CCL5/CCR5* within tumor and the related-gene polymorphisms may enhance our conclusions. Furthermore, the clinical role of

the immune environment with specific modulators such as β -arrestin2 in different sided CRC should be explored in further studies.

In conclusion, genetic variants of *CCL5* and *CCR5* SNPs may predict outcomes in mCRC patients receiving CET-based first-line treatment depending on tumor location.

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Highlights

- CCL5/CCR5 axis is involved in EGFR signaling blockade in tumor progression.
- Impact of *CCL5/CCR5* SNPs varies depending on tumor location in inhibiting EGFR.
- Cetuximab-based treatment could be a possibility in right-sided colorectal cancer.

Fig. 1.
Models combining tumor location with *CCL5*rs2280789 (Model 1) and *CCR5*rs1799988 (Model 2).

Novel classification model

Fig. 2.

A novel classification composed of *CCL5* rs2280789 and *CCR5* rs1799988 based on tumor location regarding clinical outcome in the evaluation cohort, which was finally divided into three categories: Group I, Group II and Group III.

Fig. 3.

Progression-free survival (PFS) and overall survival (OS) according to the novel classification consisting of *CCL5/CCR5* SNPs and tumor location in the evaluation cohort: (A) PFS in *KRAS* wild-type; (B) OS in *KRAS* wild-type; (C) OS in *RAS* wild-type. (D) OS by the novel classification and stratified by treatment arms in the FIRE-3 trial. CET, cetuximab; BEV, bevacizumab.

Fig. A. 1.
Role of CCL5/CCR5 axis in EGFR signaling.

Table A. 1.

Candidate SNPs in CCL5/CCR5 pathway

Genes rs number	Allele location	Base exchange	MAF [‡] (CEU)	Function of polymorphism	Forward / Reverse primer (5'–3')
<i>CCL5</i> rs2280789	Intron Chromosome 17:35879999	A>G	0.11	Transcriptional regulation	F: ATCTCCCCAACATGAGTCCA R: CCATAATGTCCTGTTGTCTTGA
<i>CCR5</i> rs1799988	5' prime UTR Chromosome 3:46370768	C>T	0.47	Transcriptional regulation	F: TGGGATGAGCAGAGAACAAA R: GCGGAAAAGAAATCAGAGAACAA

MAF, Minor allele frequency; CEU, Caucasian; F, forward primer; R, reverse primer; Tag SNP, tagging SNP.

[‡]In Caucasians from the Ensembl Genome Browser: <http://uswest.ensembl.org/index.html>.

Table A. 2.

Baseline patients and tumor characteristics

Cohort	Evaluation cohort (N=244) FIRE-3, FOLFIRI+CET arm		Control cohort (N=247) FIRE-3, FOLFIRI+BEV arm		P value *
	N	%	N	%	
Sex					
Male	169	69	162	66	0.38
Female	75	31	85	34	
Age (year)					
Median (range)	64 (38–79)		65 (31–76)		
65	130	53	130	53	0.89
> 65	114	47	117	47	
ECOG Performance status					
ECOG 0	124	51	133	54	0.50
ECOG 1–2	120	49	114	46	
Primary tumor site					
Right	45	18	62	25	0.070
Left	194	80	179	72	
Unknown	5	2	6	2	
Liver metastasis					
Yes	84	34	81	33	0.70
No	160	66	166	67	
Lung metastasis					
Yes	94	39	94	38	0.92
No	150	61	153	62	
Number of metastases					
0–1	105	43	107	43	0.95
2	139	57	140	57	
Primary tumor resected					
Yes	203	83	213	86	0.47
No	39	16	34	14	
Unknown	2	1	0	0	

Cohort	Evaluation cohort (N=244) FIRE-3, FOLFIRI+CET arm		Control cohort (N=247) FIRE-3, FOLFIRI+BEV arm		P value *
	N	%	N	%	
Adjuvant history					
Yes	50	20	45	18	0.49
No	192	79	202	82	
Unknown	2	1	0	0	
RAS status					
Wild-type	192	79	199	81	0.64
Mutant	38	16	35	14	
Unknown	14	6	13	5	
BRAF status					0.78
Wild-type	214	88	214	87	
Mutant	22	9	24	10	
Unknown	8	3	9	4	

* P value was based on Chi-square test, or the Wilcoxon test when appropriate.

Table A. 3.

Association of baseline characteristics with clinical outcome in the evaluation cohort

	N	Progression-free survival			P value *	Overall survival			P value *
		Median (95%CI), months	Univariate HR (95%CI) †	Univariate HR (95%CI) †		Median (95%CI), months	Univariate HR (95%CI) †	Univariate HR (95%CI) †	
Sex									
Male	169	10.5(9.3,12.2)	1(Reference)		0.004	30.6(23.9,38.5)	1(Reference)		0.13
Female	75	7.9(6.1,10.4)	1.52(1.14,2.04)			27.9(19.9,37.1)	1.31(0.92,1.87)		
Age (year)									
65	130	10.3(9.3,12.2)	1(Reference)		0.083	29.8(23.9,38.5)	1(Reference)		0.22
> 65	114	9.0(7.5,10.6)	1.27(0.96,1.67)			30.6(20.6,38.3)	1.23(0.87,1.74)		
ECOG Performance status									
ECOG 0	124	10.4(9.6,12.2)	1(Reference)		0.36	33.1(24.4,42.8)	1(Reference)		0.051
ECOG 1-2	120	8.8(7.6,10.5)	1.14(0.86,1.49)			26.5(20.5,36.6)	1.40(0.99,1.97)		
Primary tumor site									
Right	45	7.4(4.4,9.0)	1(Reference)		<0.001	18.5(14.9,24.5)	1(Reference)		<0.001
Left	194	10.4(9.6,11.8)	0.51(0.36,0.72)			36.6(29.8,42.8)	0.41(0.27,0.61)		
Liver metastasis									
Yes	84	10.4(8.3,12.8)	1(Reference)		0.21	37.5(24.4,56.2)	1(Reference)		0.021
No	160	9.7(8.2,10.9)	1.20(0.90,1.61)			28.0(21.7,33.6)	1.53(1.06,2.21)		
Lung metastasis									
Yes	94	9.0(7.4,10.6)	1(Reference)		0.22	27.9(18.3,36.4)	1(Reference)		0.051
No	150	10.4(9.2,11.8)	0.84(0.64,1.12)			33.1(24.4,40.9)	0.71(0.51,1.01)		
Number of metastases									
0-1	105	10.2(9.0,12.2)	1(Reference)		0.38	36.6(25.2,40.9)	1(Reference)		0.052
2	139	9.6(8.1,11.1)	1.13(0.86,1.49)			27.9(20.6,33.8)	1.40(0.99,1.98)		
Primary tumor resected									
Yes	203	9.7(8.5,10.6)	1(Reference)		0.46	30.0(24.4,38.3)	1(Reference)		0.31
No	39	10.4(5.7,14.0)	0.86(0.58,1.28)			28.0(15.3,59.0)	1.27(0.80,2.04)		
Adjuvant history									
Yes	50	9.9(7.6,12.2)	1(Reference)		0.32	33.1(21.7,51.3)	1(Reference)		0.93
No	192	9.9(8.7,10.9)	0.85(0.61,1.18)			29.8(23.7,37.1)	1.02(0.67,1.54)		

	N	Progression-free survival			Overall survival		
		Median (95%CI), months	Univariate HR (95%CI) †	P value *	Median (95%CI), months	Univariate HR (95%CI) †	P value *
<i>RAS</i> status							
Wild-type	192	10.4(9.3,12.1)	1(Reference)	<0.001	33.1(24.5,39.4)	1(Reference)	0.004
Mutant	38	6.0(5.1,8.5)	1.90(1.31,2.76)		19.1(15.9,27.6)	1.85(1.19,2.90)	
<i>BRAF</i> status							
Wild-type	214	10.2(9.2,11.3)	1(Reference)	<0.001	33.1(24.5,38.5)	1(Reference)	<0.001
Mutant	22	3.4(1.9,8.6)	2.61(1.65,4.15)		12.9(5.2,23.8)	2.69(1.60,4.52)	

P values < 0.05 were shown in bold.

* P value was based on the log-rank test for PFS and OS in the univariate analysis (†).

Table A. 4.

Association of baseline characteristics with clinical outcome in the control cohort

	N	Progression-free survival			Overall survival		
		Median (95%CI), months	Univariate HR (95%CI) †	P value *	Median (95%CI), months	Univariate HR (95%CI) †	P value *
Sex				0.16			0.64
Male	162	10.3(9.7,11.7)	1(Reference)		24.2(20.6,28.0)	1(Reference)	
Female	85	10.1(8.6,13.0)	1.23(0.92,1.63)		25.6(21.9,30.8)	0.92(0.66,1.28)	
Age (year)				0.63			0.31
65	130	10.1(9.1,12.0)	1(Reference)		23.1(19.0,28.4)	1(Reference)	
> 65	117	10.4(9.7,12.2)	1.07(0.81,1.41)		25.4(23.1,28.8)	0.85(0.62,1.17)	
ECOG Performance status				<0.001			0.001
ECOG 0	133	11.8(10.3,13.5)	1(Reference)		29.0(25.4,33.2)	1(Reference)	
ECOG 1-2	114	9.7(8.8,10.2)	1.61(1.22,2.13)		21.0(18.6,23.8)	1.66(1.21,2.27)	
Primary tumor site				0.043			0.011
Right	62	9.0(6.9,10.9)	1(Reference)		21.8(16.5,23.6)	1(Reference)	
Left	179	10.7(9.8,12.2)	0.72(0.52,1.00)		27.4(23.7,30.8)	0.64(0.45,0.91)	
Liver metastasis				0.58			0.033
Yes	81	12.0(9.7,13.1)	1(Reference)		27.6(20.6,38.1)	1(Reference)	
No	166	9.9(9.1,10.8)	1.08(0.81,1.45)		24.7(21.2,26.5)	1.43(1.02,2.01)	
Lung metastasis				0.67			0.85
Yes	94	9.9(8.9,11.5)	1(Reference)		26.1(23.2,28.4)	1(Reference)	
No	153	10.8(9.7,12.3)	1.06(0.80,1.42)		23.1(19.5,28.6)	1.03(0.74,1.43)	
Number of metastases				0.14			0.010
0-1	107	11.9(9.9,13.0)	1(Reference)		27.6(21.3,36.0)	1(Reference)	
2	140	9.8(9.0,10.8)	1.23(0.93,1.62)		23.7(21.0,26.4)	1.50(1.09,2.07)	
Primary tumor resected				0.80			0.044
Yes	213	10.1(9.6,11.5)	1(Reference)		25.4(22.3,28.4)	1(Reference)	
No	34	10.8(8.8,14.6)	0.95(0.62,1.45)		21.2(16.7,26.5)	1.60(0.99,2.58)	
Adjuvant history				0.93			0.61
Yes	45	9.7(8.8,10.8)	1(Reference)		25.6(19.4,35.0)	1(Reference)	
No	202	10.5(9.8,12.3)	1.01(0.72,1.43)		24.7(21.5,28.0)	1.11(0.75,1.63)	

	Progression-free survival			Overall survival		
	N	Median (95%CI), months	Univariate HR (95%CI) †	P value *	Median (95%CI), months	Univariate HR (95%CI) †
<i>RAS</i> status						
Wild-type	199	10.2(9.3,11.5)	1(Reference)	0.89	25.4(23.0,28.6)	1(Reference)
Mutant	35	10.9(8.9,13.9)	0.97(0.64,1.47)		19.0(16.7,26.5)	1.33(0.83,2.15)
<i>BRAF</i> status						
Wild-type	214	11.3(10.1,12.4)	1(Reference)	<0.001	27.4(23.8,29.6)	1(Reference)
Mutant	24	6.3(3.6,7.8)	3.43(2.19,5.39)		13.7(7.8,19.5)	2.74(1.74,4.31)

P values < 0.05 were shown in bold.

* P value was based on the log-rank test for PFS and OS in the univariate analysis (†).

Table A. 5.

Models consisting SNPs and tumor location in the control cohort

Tumor response					Progression-free survival				Overall Survival				
N	CR+PR	SD+PD	P * value	Median, months (95%CI)	HR (95%CI) †	P * value	HR (95%CI) †	P * value	Median, months (95%CI)	HR (95%CI) †	P * value	HR (95%CI) †	P * value
Model 1													
CCL5 by tumor location													
Group I	142	85(64%)	47(36%)	0.97	11.1(9.9,12.8)	1(Reference)	1(Reference)	0.22	27.4(22.3,33.2)	1(Reference)	0.067	1(Reference)	0.65
Group II	36	21(66%)	11(34%)		9.8(8.6,12.2)	1.10(0.73,1.65)	0.98(0.65,1.50)		28.1(19.5,31.9)	1.16(0.74,1.83)		0.97(0.60,1.55)	
Group III	45	25(63%)	15(38%)		8.8(6.6,12.5)	1.38(0.95,2.01)	1.13(0.77,1.67)		23.0(15.9,23.8)	1.58(1.04,2.39)		1.22(0.77,1.92)	
Group IV	17	9(60%)	6(40%)		9.3(5.2,12.3)	1.49(0.85,2.60)	0.93(0.51,1.70)		20.6(14.1,23.1)	1.77(0.96,3.27)		0.78(0.37,1.66)	
Model 2													
CCR5 by tumor location													
Group I	53	31(69%)	14(31%)	0.76	10.7(9.3,13.6)	1(Reference)	1(Reference)	0.20	29.6(19.5,36.0)	1(Reference)	0.075	1(Reference)	0.30
Group II	122	71(61%)	45(39%)		10.8(9.7,11.9)	1.08(0.75,1.54)	1.22(0.84,1.76)		27.4(21.9,30.3)	1.08(0.72,1.63)		1.25(0.82,1.91)	
Group III	19	11(69%)	5(31%)		8.8(6.5,12.5)	1.57(0.89,2.76)	1.58(0.89,2.82)		22.7(13.7,29.1)	1.72(0.90,3.28)		1.87(0.95,3.68)	
Group IV	41	22(59%)	15(41%)		9.3(6.6,12.3)	1.43(0.90,2.26)	1.13(0.70,1.84)		21.0(14.7,23.8)	1.67(1.01,2.76)		1.12(0.64,1.97)	
Novel classification													
Group I	152	90(65%)	48(35%)	0.73	10.8(9.9,12.7)	1(Reference)	1(Reference)	0.16	27.5(24.7,32.4)	1(Reference)	0.025	1(Reference)	0.20
Group II	43	23(61%)	15(39%)		9.7(8.3,11.5)	1.27(0.88,1.84)	1.26(0.87,1.84)		23.2(15.9,28.4)	1.48(0.97,2.25)		1.48(0.95,2.30)	
Group III	41	22(59%)	15(41%)		9.3(6.6,12.3)	1.38(0.93,2.04)	1.02(0.67,1.56)		21.0(14.7,23.8)	1.64(1.08,2.50)		1.02(0.62,1.68)	
RAS wild-type subgroup													
Model 1													
CCL5 by tumor location													
Group I	116	71(66%)	36(34%)	0.26	10.8(9.8,12.7)	1(Reference)	1(Reference)	0.42	27.5(23.7,33.2)	1(Reference)	0.14	1(Reference)	0.30
Group II	31	19(68%)	9(32%)		9.7(8.3,11.5)	1.11(0.72,1.71)	0.98(0.62,1.55)		28.0(18.4,31.9)	1.19(0.73,1.93)		1.01(0.61,1.66)	
Group III	35	19(61%)	12(39%)		8.8(6.2,12.5)	1.40(0.92,2.15)	1.12(0.72,1.73)		23.0(14.2,23.8)	1.70(1.06,2.72)		1.28(0.76,2.14)	

Tumor response				Progression-free survival				Overall Survival			
N	CR+PR	SD+PD	P value	Median, months (95%CI)	HR (95%CI)	P value	HR (95%CI)	Median, months (95%CI)	HR (95%CI)	P value	P value
Group IV	10	3(33%)	6(67%)	9.0(1.6,15.9)	1.29(0.63,2.67)	0.37	0.78(0.35,1.72)	20.6(7.1,58.7)	1.35(0.62,2.97)	0.53(0.20,1.37)	0.77
Model 2											
CCRS by tumor location				0.52		0.37				0.15	
Group I	46	25(64%)	14(36%)	10.1(8.5,12.8)	1(Reference)		1(Reference)	26.1(19.5,32.4)	1(Reference)	1(Reference)	
Group II	99	62(66%)	32(34%)	11.1(9.7,12.0)	1.02(0.69,1.49)		1.19(0.80,1.78)	28.1(24.7,33.2)	0.88(0.57,1.35)	1.12(0.71,1.77)	
Group III	14	8(67%)	4(33%)	9.0(6.5,15.7)	1.33(0.69,2.55)		1.48(0.77,2.87)	23.2(14.2,29.1)	1.29(0.61,2.72)	1.50(0.70,3.25)	
Group IV	29	13(50%)	13(50%)	7.8(5.7,12.3)	1.45(0.86,2.45)		1.07(0.61,1.87)	21.0(13.0,24.2)	1.51(0.86,2.64)	1.06(0.55,2.02)	
Novel classification				0.21		0.21				0.069	0.49
Group I	124	77(68%)	37(32%)	10.7(9.7,12.7)	1(Reference)		1(Reference)	28.8(24.8,32.4)	1(Reference)	1(Reference)	
Group II	34	18(60%)	12(40%)	9.7(8.3,11.8)	1.21(0.80,1.83)		1.24(0.82,1.89)	23.6(14.8,28.6)	1.31(0.82,2.11)	1.35(0.82,2.22)	
Group III	29	13(50%)	13(50%)	7.8(5.7,12.3)	1.46(0.92,2.30)		0.99(0.60,1.64)	21.0(13.0,24.2)	1.70(1.05,2.77)	1.03(0.57,1.87)	

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. *P* values<0.05 were shown in bold.

* *P* value was based on the Fisher's exact test for response, log-rank test in the univariate analysis

(†) and Wald test in the multivariable analysis within Cox regression model

(‡) adjusted for sex, ECOG performance status, liver metastasis, resection of the primary tumors, RAS and BRAF status.

Table 1.

Association between gene polymorphism and clinical outcome

Tumor response					Progression-free survival					Overall Survival				
N	CR+PR	SD+PD	P * value	Median, months (95%CI)	HR (95%CI) [†]	P * value	HR (95%CI) [†] ‡	P * value	Median, months (95%CI)	HR (95%CI) [†]	P * value	HR (95%CI) [†] ‡	P * value	
Evaluation cohort														
CCL5			0.21			0.22		0.23			0.024		0.030	
rs2280789														
A/A	123 (73%)	45 (27%)		10.3 (9.0, 11.1)	1 (Reference)		1 (Reference)		33.4 (27.1, 38.5)	1 (Reference)		1 (Reference)		
A/G ^a	42	23 (68%)		9.2 (6.1, 12.2)	1.23 (0.88, 1.72)		1.24 (0.87, 1.76)		19.9 (16.8, 25.2)	1.56 (1.05, 2.30)		1.57 (1.04, 2.35)		
G/G ^a	8	2 (40%)												
CCR5			0.049			0.25		0.13			0.59		0.32	
rs1799988														
C/C	65	42 (81%)		10.6 (9.2, 13.3)	1 (Reference)		1 (Reference)		36.4 (21.8, 44.1)	1 (Reference)		1 (Reference)		
C/T	107	59 (63%)		9.0 (7.5, 10.8)	1.32 (0.94, 1.84)		1.39 (0.98, 1.96)		28.7 (20.4, 38.7)	1.19 (0.78, 1.81)		1.30 (0.85, 1.98)		
T/T	62	41 (76%)		10.5 (8.2, 13.3)	1.22 (0.83, 1.80)		1.41 (0.94, 2.11)		26.5 (19.9, 37.1)	1.26 (0.79, 2.00)		1.42 (0.88, 2.30)		
C/C	65	42 (81%)	0.078			0.11		0.043			0.31		0.15	
Any T	169	100 (68%)		9.4 (8.0, 10.9)	1.28 (0.94, 1.75)		1.40 (1.01, 1.93)		27.6 (22.5, 33.6)	1.21 (0.83, 1.79)		1.34 (0.90, 1.98)		
Control cohort														
CCL5			0.95			0.47		0.79			0.32		0.56	
rs2280789														
A/A	191	113 (64%)		10.5 (9.7, 12.0)	1 (Reference)		1 (Reference)		24.8 (22.3, 28.8)	1 (Reference)		1 (Reference)		
A/G ^a	48	27 (63%)		9.8 (8.9, 11.5)	1.13 (0.81, 1.57)		0.95 (0.67, 1.36)		23.1 (19.0, 28.4)	1.20 (0.83, 1.74)		0.89 (0.59, 1.33)		
G/G ^a	7	4 (67%)												
CCR5			0.38			0.84		0.39			0.72		0.97	
rs1799988														

	Tumor response			Progression-free survival					Overall Survival				
	N	CR+PR	SD+PD	P value [*]	Median, months (95%CI)	HR (95%CI) [†]	P value [*]	HR (95%CI) [‡]	P value [*]	HR (95%CI) [†]	P value [*]	HR (95%CI) [‡]	P value [*]
C/C	79	46 (68%)	22 (32%)		10.5 (9.6, 13.1)	1 (Reference)		1 (Reference)		1 (Reference)		1 (Reference)	
C/T	104	57 (58%)	41 (42%)		10.3 (9.2, 11.9)	1.10 (0.80, 1.52)		1.25 (0.90, 1.74)		0.96 (0.67, 1.37)		0.98 (0.68, 1.42)	
T/T	58	36 (67%)	18 (33%)	0.45	9.9 (8.8, 11.7)	1.07 (0.74, 1.56)	0.57	1.21 (0.83, 1.79)	0.17	0.84 (0.55, 1.30)	0.61	1.03 (0.66, 1.60)	0.97
C/C	79	46 (68%)	22 (32%)		10.5 (9.6, 13.1)	1 (Reference)		1 (Reference)		1 (Reference)		1 (Reference)	
Any T	162	93 (61%)	59 (39%)		10.2 (9.2, 11.7)	1.09 (0.81, 1.47)		1.24 (0.91, 1.68)		0.92 (0.66, 1.28)		0.99 (0.71, 1.40)	

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. *P* values < 0.05 were shown in bold.

^{*} *P* value was based on the Fisher's exact test for response, log-rank test in the univariate analysis

([†]) and Wald test in the multivariable analysis within Cox regression model

([‡]) adjusted for sex, ECOG performance status, liver metastasis, resection of the primary tumors, RAS and BRAF status.

^d Grouped together for estimates of HR.

Table 2.

Gene polymorphism and clinical outcome by tumor location in the evaluation cohort

Tumor response					Progression-free survival				Overall Survival				
N	CR+PR	SD+PD	P value*	Median, months (95%CI)	HR (95%CI) [†]	P value*	HR (95%CI) [†]	P value*	Median, months (95%CI)	HR (95%CI) [†]	P value*	HR (95%CI) [†]	P value*
Right-sided subgroup													
CCL5 rs2280789			0.25			0.93		0.54			0.35		0.14
A/A	33	19(68%)	9 (32%)	7.8 (5.3,9.5)	1 (Reference)		1 (Reference)		21.9(11.7, 27.1)	1 (Reference)		1 (Reference)	
Any G	11	4 (44%)	5 (56%)	3.9 (1.4,14.0)	1.03 (0.51, 2.09)		1.30 (0.56, 2.99)		18.3 (10.1,18.9)	1.42 (0.64, 3.17)		2.01 (0.80, 5.02)	
CCR5 rs 17 99988			0.012			0.072		0.058			0.022		0.26
C/C	8	2 (25%)	6 (75%)	5.5 (1.4,14.2)	1 (Reference)		1 (Reference)		13.7 (5.5, 21.9)	1 (Reference)		1 (Reference)	
C/T	22	10(59%)	7 (41%)	7.2 (2.5,9.0)	1.34 (0.57, 3.17)		1.93 (0.75, 4.98)		16.1 (7.1, 27.9)	0.67 (0.27,1.67)		0.60(0.23,1.54)	
T/T	14	11(92%)	1(8%)	9.0 (5.9,14.1)	0.64 (0.26,1.57)		0.64 (0.21, 1.97)		27.1 (16.4, 37.5)	0.31 (0.11,0.88)		0.38 (0.12,1.22)	
Any C	30	12 (48%)	13 (52%)	6.5 (3.0,8.8)	1 (Reference)	0.037	1 (Reference)	0.052	15.8 (10.3,18.9)	1 (Reference)	0.013	1 (Reference)	0.22
T/T	14	11(92%)	1(8%)	9.0 (5.9,14.1)	0.52 (0.26,1.05)		0.41(0.17, 1.01)		27.1 (16.4, 37.5)	0.41(0.18,0.91)		0.54 (0.20,1.46)	
Left-sided subgroup													
CCL5 rs2280789			0.82			0.44		0.42			0.14		0.19
A/A	157	103 74%)	36 (26%)	10.5 (9.6,12.2)	1 (Reference)		1 (Reference)		38.3 (30.6, 44.1)	1 (Reference)		1 (Reference)	
Any G	36	20(71%)	8 (29%)	10.0 6.9,13.5)	1.17 (0.78,1.74)		1.19(0.78,1.82)		21.7(18.1, 46.3)	1.43 (0.89,2.31)		1.39(0.85,2.28)	
CCR5 rs1799988			0.003			0.27		0.14			0.32		0.23
C/C	56	40 (91%)	4(9%)	10.6 (9.9,14.1)	1 (Reference)		1 (Reference)		38.5 (33.1, 49.8)	1 (Reference)		1 (Reference)	
C/T	84	49 (64%)	28 (36%)	10.2 (7.7,12.2)	1.29 (0.89,1.88)		1.35(0.92,1.98)		33.4 (23.7, 52.0)	1.23 (0.75,1.99)		1.36(0.83,2.24)	
T/T	45	28 (72%)	11 (28%)	9.6 (6.5,14.0)	1.36 (0.87, 2.13)		1.56(0.98,2.49)		27.6(17.3,41.2)	1.52 (0.88,2.64)		1.63(0.92,2.90)	
C/C	56	40 (91%)	4(9%)	10.6 (9.9,14.1)	1 (Reference)	0.11	1 (Reference)	0.063	38.5 (33.1, 49.8)	1 (Reference)	0.22	1 (Reference)	0.12

Tumor response				Progression-free survival				Overall Survival			
N	CR+PR	SD+PD	P value*	Median, months (95%CI)	HR (95%CI) [†]	P value*	HR (95%CI) [†]	P value*	Median, months (95%CI)	HR (95%CI) [†]	P value*
Any T	129	77(66%)	39 (34%)	10.0 (8.2,12.1)	1.32 (0.93,1.87)		1.41(0.98,2.01)		30.6 (22.6, 40.9)	1.32 (0.84,2.07)	
Combined tumor site and SNPs models											
Model 1											
CCL5 by tumor location				0.28		0.001		0.008			<0.001
Group I	157	103 (74%)	36 (26%)	10.5 (9.6,12.2)	1 (Reference)		1 (Reference)		38.3 (30.6, 44.1)	1 (Reference)	
Group II	36	20(71%)	8 (29%)	10.0 (6.9,13.5)	1.17 (0.78,1.74)		1.16 (0.76,1.76)		21.7(18.1, 46.3)	1.43 (0.89,2.31)	
Group III	33	19(68%)	9 (32%)	7.8 (5.3,9.5)	1.95 (1.30, 2.91)		1.75 (1.15, 2.66)		21.9(11.7, 27.1)	2.45 (1.55, 3.88)	
Group IV	11	4 (44%)	5 (56%)	3.9 (1.4,14.0)	2.13(1.14,3.96)		2.27(1.21,4.26)		18.3 (10.1, 18.9)	3.33(1.62,6.84)	
Model 2											
CCR5 by tumor location				<0.001		<0.001		<0.001			<0.001
Group I	56	40 (91%)	4(9%)	10.6 (9.9,14.1)	1 (Reference)		1 (Reference)		38.5 (33.1, 49.8)	1 (Reference)	
Group II	129	77(66%)	39 (34%)	10.0 (8.2,12.1)	1.30 (0.92,1.84)		1.41 (0.99, 2.02)		30.6(22.6, 40.9)	1.32 (0.84,2.06)	
Group III	14	11(92%)	1(8%)	9.0 (5.9,14.1)	1.56 (0.84, 2.91)		1.67 (0.88, 3.19)		27.1 (16.4, 37.5)	1.66 (0.78, 3.53)	
Group IV	30	12 (48%)	13 (52%)	6.5 (3.0,8.8)	3.00(1.85,4.86)		2.87 (1.74, 4.72)		15.8(10.3,18.9)	3.99 (2.26, 7.06)	
Novel classification				0.020		<0.001		0.001			<0.001
Group I	161	104 (75%)	35 (25%)	10.6 (9.5,12.2)	1 (Reference)		1 (Reference)		38.3 (30.6, 44.1)	1 (Reference)	
Group II	37	23(72%)	9 (28%)	10.0 (6.9,13.4)	1.20 (0.82,1.75)		1.23 (0.81,1.85)		24.4(18.1, 37.1)	1.67(1.06,2.62)	
Group III	29	11(46%)	13 (54%)	7.1 (2.9,9.0)	2.51 (1.64, 3.84)		2.26 (1.46, 3.51)		15.8 (10.3, 18.9)	3.65 (2.25, 5.92)	
RAS wild-type subgroup											
Model 1											
CCL5 by tumor location				0.059		<0.001		0.016			<0.001
Group I	122	89 (82%)	20 (18%)	10.9 (9.9,13.3)	1 (Reference)		1 (Reference)		41.2 (33.8, 49.8)	1 (Reference)	
Group II	29	16 (73%)	6 (27%)	10.0 (6.9,14.1)	1.13(0.72,1.78)		1.05 (0.65,1.69)		20.3 (16.8, 46.3)	1.79 (1.06, 3.03)	
Group III	28	15 (65%)	8 (35%)	7.3 (4.4,9.5)	2.23 (1.43, 3.49)		1.91 (1.20, 3.04)		16.5 (10.3, 27.1)	3.04 (1.83, 5.05)	
Group IV	7	2 (40%)	3 (60%)	3.9 (1.4, 15.0)	2.10 (0.97, 4.55)		2.16 (0.98, 4.77)		18.3 (2.5, 18.9)	5.40 (2.18, 13.36)	
Model 2											
Group I	122	89 (82%)	20 (18%)	10.9 (9.9,13.3)	1 (Reference)		1 (Reference)		41.2 (33.8, 49.8)	1 (Reference)	
Group II	29	16 (73%)	6 (27%)	10.0 (6.9,14.1)	1.13(0.72,1.78)		1.05 (0.65,1.69)		20.3 (16.8, 46.3)	1.79 (1.06, 3.03)	
Group III	28	15 (65%)	8 (35%)	7.3 (4.4,9.5)	2.23 (1.43, 3.49)		1.91 (1.20, 3.04)		16.5 (10.3, 27.1)	3.04 (1.83, 5.05)	
Group IV	7	2 (40%)	3 (60%)	3.9 (1.4, 15.0)	2.10 (0.97, 4.55)		2.16 (0.98, 4.77)		18.3 (2.5, 18.9)	5.40 (2.18, 13.36)	

Tumor response				Progression-free survival				Overall Survival			
N	CR+PR	SD+PD	P value*	Median, months (95%CI)	HR (95%CI) [†]	P value*	HR (95%CI) [‡]	P value*	HR (95%CI) [‡]	P value*	
CCR5 by tumor location											
Group I	46	35 (92%)	3 (8%)	11.5 (9.9, 14.0)	1 (Reference)	<0.001	1 (Reference)	0.009	1 (Reference)	<0.001	
Group II	99	66 (75%)	22 (25%)	10.5 (9.3, 13.6)	1.13 (0.77, 1.67)		1.17 (0.78, 1.75)		1.13 (0.68, 1.87)	1.18 (0.70, 1.97)	
Group III	10	7 (88%)	1 (13%)	12.2 (1.9, 18.3)	1.41 (0.68, 2.94)		1.61 (0.77, 3.39)		1.51 (0.61, 3.70)	2.17 (0.85, 5.49)	
Group IV	24	9 (47%)	10 (53%)	6.5 (2.9, 9.0)	3.12 (1.81, 5.37)		2.54 (1.43, 4.50)		4.73 (2.53, 8.86)	3.97 (2.08, 7.59)	
Novel classification											
Group I	125	90 (83%)	19 (17%)	11.1 (10.0, 13.3)	1 (Reference)	<0.001	1 (Reference)	0.005	1 (Reference)	<0.001	
Group II	29	17 (71%)	7 (29%)	10.3 (6.9, 14.0)	1.19 (0.77, 1.84)		1.16 (0.72, 1.86)		1.94 (1.16, 3.24)	2.41 (1.38, 4.23)	
Group III	24	9 (47%)	10 (53%)	6.5 (2.9, 9.0)	2.95 (1.83, 4.76)		2.33 (1.40, 3.89)		4.86 (2.83, 8.34)	3.83 (2.16, 6.81)	

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. *P* values<0.05 were shown in bold.

* *P* value was based on the Fisher's exact test for response, log-rank test in the univariate analysis

([†]) and Wald test in the multivariable analysis within Cox regression model

([‡]) adjusted for sex, ECOG performance status, liver metastasis, resection of the primary tumors, RAS and BRAF status.