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Original Research

Role of *CCL5* and *CCR5* gene polymorphisms in epidermal growth factor receptor signalling blockade in metastatic colorectal cancer: analysis of the FIRE-3 trial

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Abstract ***Background:*** Epidermal growth factor receptor signalling blockade increases CCL5 expression that regulates either the anti-tumour immune response or tumour 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 analysed by polymerase chain reaction–based direct sequencing.

Results: Patients in the evaluation cohort with any *CCL5* rs2280789G allele had shorter overall survival (OS) compared with those with the A/A variant (hazard ratio 1.56, $P = 0.024$). Patients carrying any *CCR5* rs1799988T 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 tumour 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

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follows: median OS, 38.5, 30.6, 27.1 and 15.8 months, $P < 0.001$; response rate, 91, 66, 92 and 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 and 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 tumour location.

1. Introduction

Previously reported randomised 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 tumour molecular subtype and primary tumour location, recent guidelines suggest that anti-EGFR antibodies cetuximab (CET) and panitumumab are preferred in *RAS* wild-type tumours 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 tumour location [3,4]. However, how to predict efficacy of the biologic agents has not been sufficiently clarified to date.

EGFR signalling blockade increases C–C motif chemokine ligand-5 (CCL5) expression, which attracts tumour-infiltrating leukocytes that regulates either the host-derived anti-tumour immunity or tumour progression [5,6,7]. A high degree of T cell infiltration in cancer tissue is known to be associated with a favourable prognosis in colorectal cancer. T helper type 1 expresses C–C motif chemokine receptor-5 (CCR5) and C-X-C motif chemokine receptor-3 (CXCR3), whereas T helper type 2 expresses CCR4; and CCR5-and CXCR3-expressing T cells are recruited into the invasive margin as anti-tumour immune responses. CCL5 is expressed and localised within CD8+ T cells, while a CXCR3 ligand CXCL10/IP-10 is localised in tumour cells and macrophages within the invasive margin [8]. Stimulation of EGFR downregulates CCL2, CCL5 and IP-10, while it increases CXCL8/IL-8 expression in normal cells. Conversely, EGFR signalling blockade produces opposite effects, with increased CCL2, CCL5 and IP-10 and reduced IL-8 expression. Inhibition of EGFR signalling might exert antitumor activity by favouring the recruitment of inflammatory cells and a more pronounced anti-tumour immune response, along with downregulation of IL-8, which is an important growth factor for malignant epithelial cells [6,7]. Expression of IL-8/CXCR2 in the tumour microenvironment has been shown to play a critical role in progression and metastases with increased tumour 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 tumour's ability to beat antitumor CD8+ T cells by forming infiltration of T-regular cells [11]. Nevertheless, it still remains unclear how EGFR signalling blockade regulate either the host-derived anti-tumour immunity or tumour progression via CCL5 activation in colorectal cancer patients.

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

2. Materials and methods

2.1. Study design and patients

Two different cohorts with *KRAS* exon 2 wild-type mCRC from the randomised phase III FIRE-3 trial [1] were investigated in this study: an evaluation cohort of 244 patients receiving CET plus FOLFIRI and a control cohort of 247 patients receiving bevacizumab plus FOLFIRI. All patients fulfilled the eligibility 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/m^2 followed by 250 mg/m^2 thereafter) and bevacizumab (5 mg/kg) were administered weekly and every 2 weeks, respectively. FOLFIRI (irinotecan 180 mg/m^2, 5-FU bolus 400 mg/m^2, 5-FU infusion 2400 mg/m^2 and leucovorin 200 mg/m^2) was administered every 2 weeks. Treatment was continued until any of the following occurred: disease progression, unmanageable toxicity or patient refusal. The present 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 guidelines.

2.2. Selection of candidate single-nucleotide polymorphisms

The two candidate single-nucleotide polymorphisms (SNPs) of genes *CCL5* and *CCR5* were selected using any of the following criteria: (1) SNP with biological significance according to a published literature review, (2) tagging SNPs selected by the HapMap genotype data with r^2 threshold = 0.8: http://snpinfo.niehs.nih.gov/snpinfo/snptag.php, or (3) 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 SNP (F-SNP) database: http://compbio.cs.queensu.ca/F-SNP/ (Table A. 1).

2.3. 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 polymerase chain reaction (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 analysed by direct DNA sequencing for each SNP, and the genotype concordance rate was of 99% or more. The investigators analysing SNPs were blinded to the clinical data.

2.4. Statistical analysis

The primary end-point of the present study was progression-free survival (PFS), and secondary end-points 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 the 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 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 were 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 tumour site, then built the models combining tumour 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 1.49–1.65 for an SNP with minor allele frequency of 0.1–0.5 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 of 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 two-sided at a significance level of 0.050.

3. Results

3.1. Baseline patients and tumour characteristics

The median follow-up time, median PFS and OS were 34.1, 9.8 and 29.7 months in the evaluation cohort; and 39.4, 10.2 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 Tables A. 3 and A. 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 Eastern Cooperative Oncology Group (ECOG) performance status, 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 (Broad Institute, Cambridge, MA, USA).

3.2. Association of clinical outcome and SNPs tested in the evaluation cohort

In the evaluation cohort, patients with any *CCL5* rs2280789G allele had shorter OS compared to those

Table 1
Association between gene polymorphism and clinical outcome.

	N	Tumour response			Progression-free survival					Overall Survival				
		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 rs2280789				0.21			0.22		0.23			**0.024**		**0.030**
A/A	192	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%)	11 (32%)		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%)	3 (60%)											
CCR5 rs1799988				**0.049**			0.25		0.13			0.59		0.32
C/C	65	42 (81%)	10 (19%)		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%)	35 (37%)		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%)	13 (24%)		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)	
				0.078			**0.11**		**0.043**			0.31		0.15
C/C	65	42 (81%)	10 (19%)		10.6 (9.2, 13.3)	1 (Reference)		1 (Reference)		36.4 (21.8, 44.1)	1 (Reference)		1 (Reference)	
Any T	169	100 (68%)	48 (32%)		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 rs2280789				0.95			0.47		0.79			0.32		0.56
A/A	191	113 (64%)	63 (36%)		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%)	16 (37%)		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%)	2 (33%)											
CCR5 rs1799988				0.38			0.84		0.39			0.72		0.97
C/C	79	46 (68%)	22 (32%)		10.5 (9.6, 13.1)	1 (Reference)		1 (Reference)		23.8 (20.1, 27.6)	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)		24.8 (19.4, 28.1)	0.96 (0.67, 1.37)		0.98 (0.68, 1.42)	
T/T	58	36 (67%)	18 (33%)		9.9 (8.8, 11.7)	1.07 (0.74, 1.56)		1.21 (0.83, 1.79)		28.6 (22.3, 34.6)	0.84 (0.55, 1.30)		1.03 (0.66, 1.60)	
				0.45			0.57		0.17			0.61		0.97
C/C	79	46 (68%)	22 (32%)		10.5 (9.6, 13.1)	1 (Reference)		1 (Reference)		23.8 (20.1, 27.6)	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)		25.4 (21.5, 28.8)	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 tumours, RAS and BRAF status.
[a] Grouped together for estimates of HR.

Table 2
Gene polymorphism and clinical outcome by tumour location in the evaluation cohort.

		Tumour 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 rs1799988				**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)	
				0.013			**0.037**		**0.052**			**0.013**		0.22
Any C	30	12 (48%)	13 (52%)		6.5 (3.0, 8.8)	1 (Reference)		1 (Reference)		15.8 (10.3, 18.9)	1 (Reference)		1 (Reference)	
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)	
				0.001			0.11		0.063			0.22		0.12
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)	
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)		1.45 (0.91,2.29)	
Combined tumour site and SNPs models														
Model 1														
CCL5 by tumour location				0.28			**0.001**		**0.008**			**< 0.001**		**< 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)		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)		1.45 (0.89, 2.37)	
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)		2.33 (1.45, 3.74)	
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)		3.11 (1.49, 6.50)	
Model 2														
CCR5 by tumour location				**< 0.001**			**< 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)		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)		1.48 (0.94, 2.34)	
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)		2.06 (0.93, 4.55)	
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)		3.86 (2.14, 6.97)	
Novel classification				**0.020**			**< 0.001**		**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)		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)		1.91 (1.16, 3.13)	
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)		3.26 (1.95, 5.42)	
***RAS* wild-type subgroup**														

Model 1												
CCL5 by tumour location				0.059			**< 0.001**		**0.016**		**< 0.001**	**< 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)	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)	1.98 (1.14, 3.42)
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)	2.87 (1.70, 4.85)
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)	4.63 (1.88, 11.42)
Model 2												
CCR5 by tumour location				**0.002**			**< 0.001**		**0.009**		**< 0.001**	**< 0.001**
Group I	46	35 (92%)	3 (8%)		11.5 (9.9, 14.0)	1 (Reference)		1 (Reference)		42.8 (33.8, 55.5)	1 (Reference)	1 (Reference)
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)		38.7 (24.4, 52.0)	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)		27.1 (5.9, 60.7)	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)		11.7 (7.1, 18.5)	4.73 (2.53, 8.86)	3.97 (2.08, 7.59)
Novel classification				**0.005**			**< 0.001**		**0.005**		**< 0.001**	**< 0.001**
Group I	125	90 (83%)	19 (17%)		11.1 (10.0, 13.3)	1 (Reference)		1 (Reference)		40.9 (33.4, 51.3)	1 (Reference)	1 (Reference)
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)		19.9 (16.8, 37.1)	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)		11.7 (7.1, 18.5)	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 tumours, RAS and BRAF status.

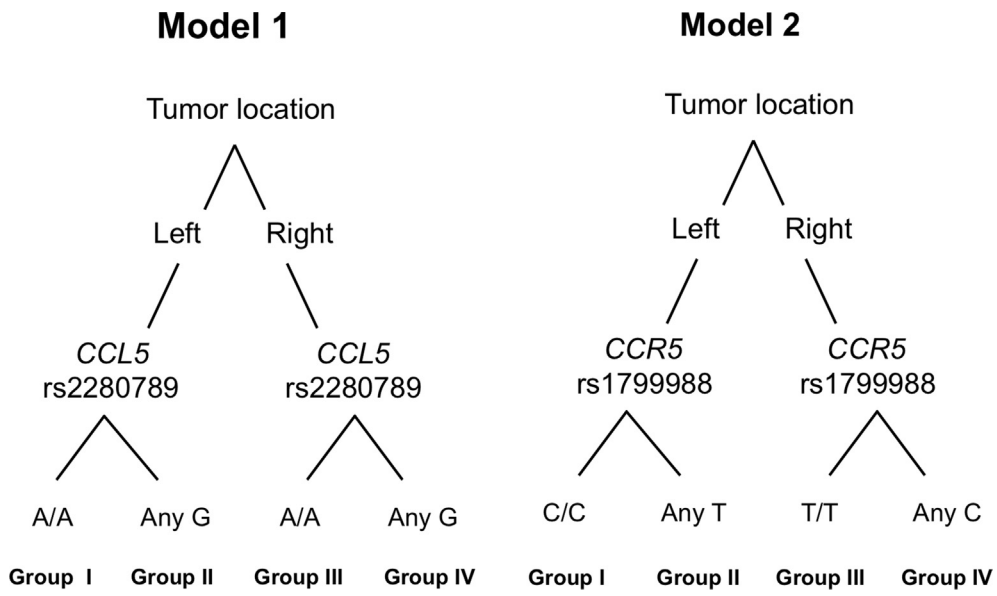

Fig. 1. Models combining tumour location with *CCL5* rs2280789 (model 1) and *CCR5* rs1799988 (model 2).

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* rs1799988T 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).

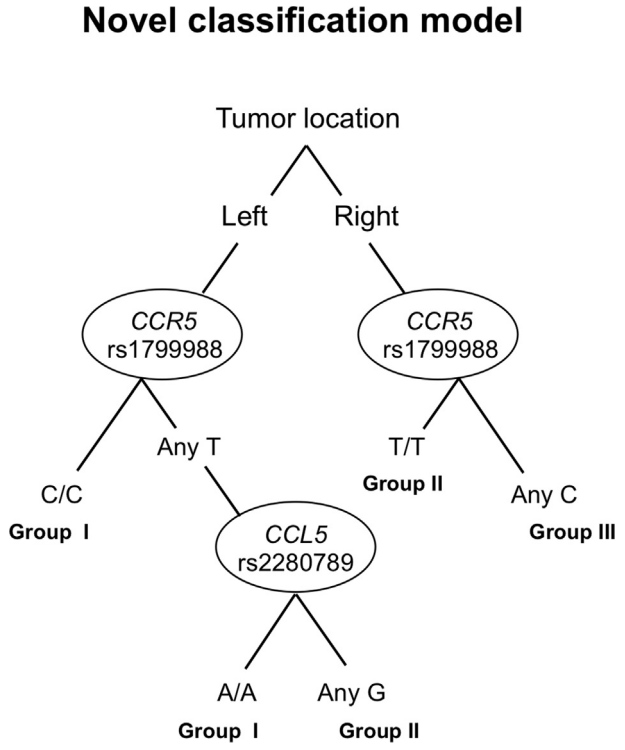

Fig. 2. A novel classification composed of *CCL5* rs2280789 and *CCR5* rs1799988 based on tumour location regarding clinical outcome in the evaluation cohort, which was finally divided into three categories: Group I, Group II and Group III.

Subgroup analysis by primary tumour location showed that patients with any *CCL5* rs2280789G 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 tumours; the T/T variant was favourable in right-sided tumours, while T allele was unfavourable in left-sided tumours for tumour response, PFS and OS (Table 2). The phenomena were clearly identified in models combining SNPs and tumour location (Fig. 1). Statistically significant differences were shown among the groups consisting of primary tumour 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* rs2280789G allele was associated with poor tumour response, shorter PFS and OS compared with the A/A variant regardless of tumour location, suggesting similar allelic characteristics between tumour locations. In model 2, patients with right-sided tumour and any *CCR5* C allele (Group IV) had poor tumour response, shorter PFS and OS compared with those with a left-sided tumour and the *CCR5* C/C variant (Group I), suggesting an opposite allelic effects between tumour locations (Table 2).

We further developed a novel classification model composed of tumour 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 tumour response, PFS and OS were observed among the three categories in both *KRAS* and *RAS* wild-type populations (Table 2, Fig. 3A–C).

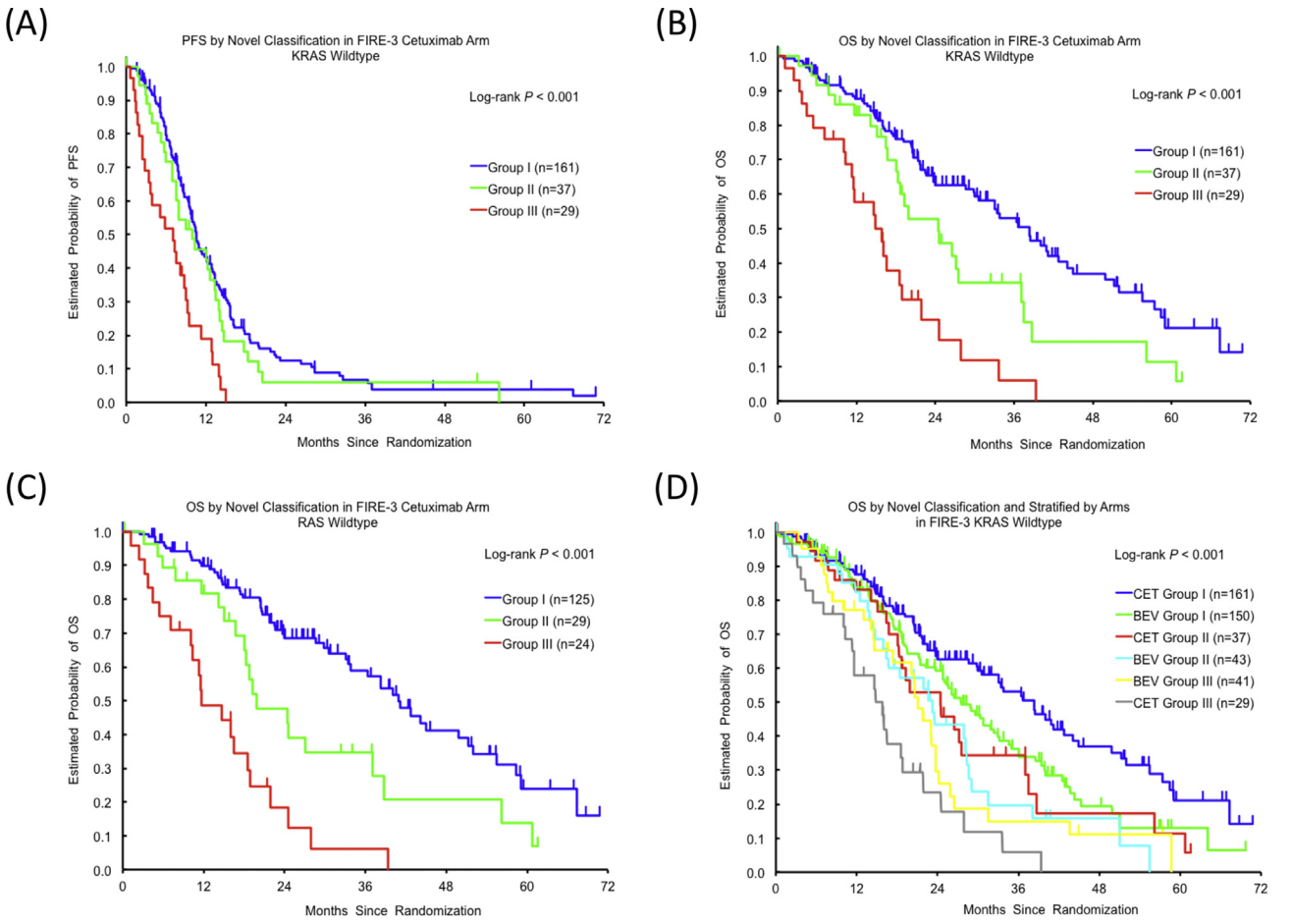

Fig. 3. Progression-free survival (PFS) and overall survival (OS) according to the novel classification consisting of *CCL5*/*CCR5* SNPs and tumour location in the evaluation cohort: (A) PFS in *KRAS* wild-type; (B) OS in *KRAS* wild-type; (C) OS in *RAS* wild-type. (D) OS by the novel classification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

3.3. 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.

4. Discussion

Our data show the first evidence that SNPs of *CCL5* and *CCR5* genes are associated with clinical outcomes in first-line CET plus FOLFIRI in patients with mCRC. In addition, the novel classification suggests that CET-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 tumour progression through recruitment of specific immune cells by regulating either the host-derived anti-tumour immunity or tumour progression along with the concomitant chemokines [5,13,14]. An RNA expression analysis study of chemokines and chemokine receptors using real-time 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 tumour-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 localised on immune cells [15]. We thus expected that the CCL5/CCR5 signalling 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 [16,17,18,19,20,21,22,23]. 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 tumour location, whereas *CCL5* SNPs showed a similar trend regardless of tumour location. Furthermore, both the SNPs and primary tumour location had greater influence on OS than PFS. Considering not significant difference in PFS between CET 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 randomised 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 tumour shrinkage, however, which still continues to confuse physician in treatment selection for *RAS* wild-type right-sided mCRC. In terms of tumour shrinkage, our novel classification could identify more clearly who benefit from chemotherapy plus EGFR antibody than tumour 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 tumour location were not completely matching each other, and our novel classification seems to cover the ambiguity of primary tumour location in predicting tumour 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, that is, 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 tumour 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 desensitised or internalised. 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 categorised as microsatellite instability (MSI) immune harbouring immune infiltration and activation. CMS1 has been shown to localise 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 tumours may account for the diverse manner of *CCR5* SNPs in the mechanism of action of CET. Moreover, in a subgroup analysis of a randomised Phase III trial (CALGB/SWOG80405) comparing CET-based to BEV-based first-line chemotherapy, CMS1 was also frequently shown in right-sided tumours and revealed to be a potentially negative predictor of CET [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 cross-talk between EGFR signalling 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 CET 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 are warranted [33].

The strengths of our study are as follows: the two cohorts derived from a randomised phase III trial (FIRE-3) comparing CET-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 tumour location for clinical outcome in CET-based treatment. In addition, correlation between expression of CCL5/CCR5 within tumour 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 tumour location.

Conflicts of interest statement

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

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Appendix

Table A 2
Baseline patients and tumour 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 (years)			
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 tumour 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 tumour resected			
Yes	203 (83)	213 (86)	0.47
No	39 (16)	34 (14)	
Unknown	2 (1)	0 (0)	
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 1
Candidate SNPs in CCL5/CCR5 pathway.

Genes rs number	Allele location	Base exchange	MAF* (CEU)	Function of polymorphism	Forward/reverse primer (5′-3′)
CCL5 rs2280789	Intron chromosome 17:35879999	A>G	0.11	Transcriptional regulation	F: ATCTCCCCAACATGAGTCCA R: CCATATGTCCTGTTGTCCTTGA
CCR5 rs1799988	5′ prime UTR chromosome 3:46370768	C>T	0.47	Transcriptional regulation	F: TGGGATGAGCAGAGAACAAA R: GGCGAAAAGAATCAGAGAACA

CEU, Caucasian; F, forward primer; MAF, minor allele frequency; R, reverse primer; Tag SNP, tagging single-nucleotide polymorphism.
* In Caucasians from the Ensembl genome browser: http://uswest.ensembl.org/index.html.

Table A 3
Association of baseline characteristics with clinical outcome in the evaluation cohort.

		Progression-free survival			Overall survival		
	N	Median (95%CI), months	Univariate HR (95%CI) †	*P* value*	Median (95%CI), months	Univariate HR (95%CI) †	*P* value*
Sex				**0.004**			0.13
Male	169	10.5 (9.3,12.2)	1(Reference)		30.6 (23.9,38.5)	1(Reference)	
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)				0.083			0.22
≤ 65	130	10.3 (9.3,12.2)	1(Reference)		29.8 (23.9,38.5)	1(Reference)	
> 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				0.36			0.051
ECOG 0	124	10.4 (9.6,12.2)	1(Reference)		33.1 (24.4,42.8)	1(Reference)	
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 tumour site				**< 0.001**			**< 0.001**
Right	45	7.4 (4.4,9.0)	1(Reference)		18.5 (14.9,24.5)	1(Reference)	
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				0.21			**0.021**
Yes	84	10.4 (8.3,12.8)	1(Reference)		37.5 (24.4,56.2)	1(Reference)	
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				0.22			0.051
Yes	94	9.0 (7.4,10.6)	1(Reference)		27.9 (18.3,36.4)	1(Reference)	
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				0.38			0.052
0-1	105	10.2 (9.0,12.2)	1(Reference)		36.6 (25.2,40.9)	1(Reference)	
≥ 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 tumour resected				0.46			0.31
Yes	203	9.7 (8.5,10.6)	1(Reference)		30.0 (24.4,38.3)	1(Reference)	
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				0.32			0.93
Yes	50	9.9 (7.6,12.2)	1(Reference)		33.1 (21.7,51.3)	1(Reference)	
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)	
RAS status				**< 0.001**			**0.004**
Wild-type	192	10.4 (9.3,12.1)	1(Reference)		33.1 (24.5,39.4)	1(Reference)	
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)	
BRAF status				**< 0.001**			**< 0.001**
Wild-type	214	10.2 (9.2,11.3)	1(Reference)		33.1 (24.5,38.5)	1(Reference)	
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)	

CI, confidence interval; HR, hazard ratio. *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.

		Progression-free survival			Overall survival		
	N	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 tumour 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 tumour 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)	
RAS status				0.89			0.23
Wild-type	199	10.2 (9.3,11.5)	1(Reference)		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)	
BRAF status				**< 0.001**			**< 0.001**
Wild-type	214	11.3 (10.1,12.4)	1(Reference)		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)	

CI, confidence interval; HR, hazard ratio. *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.

	N	Tumor response			Progression-free survival					Overall Survival				
		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				0.97			0.22		0.90			0.067		0.65
Group I	142	85 (64%)	47 (36%)		11.1 (9.9,12.8)	1(Reference)		1(Reference)		27.4 (22.3,33.2)	1(Reference)		1(Reference)	
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				0.76			0.20		0.46			0.075		0.30
Group I	53	31 (69%)	14 (31%)		10.7 (9.3,13.6)	1(Reference)		1(Reference)		29.6 (19.5,36.0)	1(Reference)		1(Reference)	
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				0.73			0.16		0.47			**0.025**		0.20
Group I	152	90 (65%)	48 (35%)		10.8 (9.9,12.7)	1(Reference)		1(Reference)		27.5 (24.7,32.4)	1(Reference)		1(Reference)	
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				0.26			0.42		0.85			0.14		0.30
Group I	116	71 (66%)	36 (34%)		10.8 (9.8,12.7)	1(Reference)		1(Reference)		27.5 (23.7,33.2)	1(Reference)		1(Reference)	
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)	
Group IV	10	3 (33%)	6 (67%)		9.0 (1.6,15.9)	1.29 (0.63,2.67)		0.78 (0.35,1.72)		20.6 (7.1,58.7)	1.35 (0.62,2.97)		0.53 (0.20,1.37)	
Model 2														
CCR5 by tumor location				0.52			0.37		0.66			0.15		0.77
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		0.58			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 tumours, RAS and BRAF status.

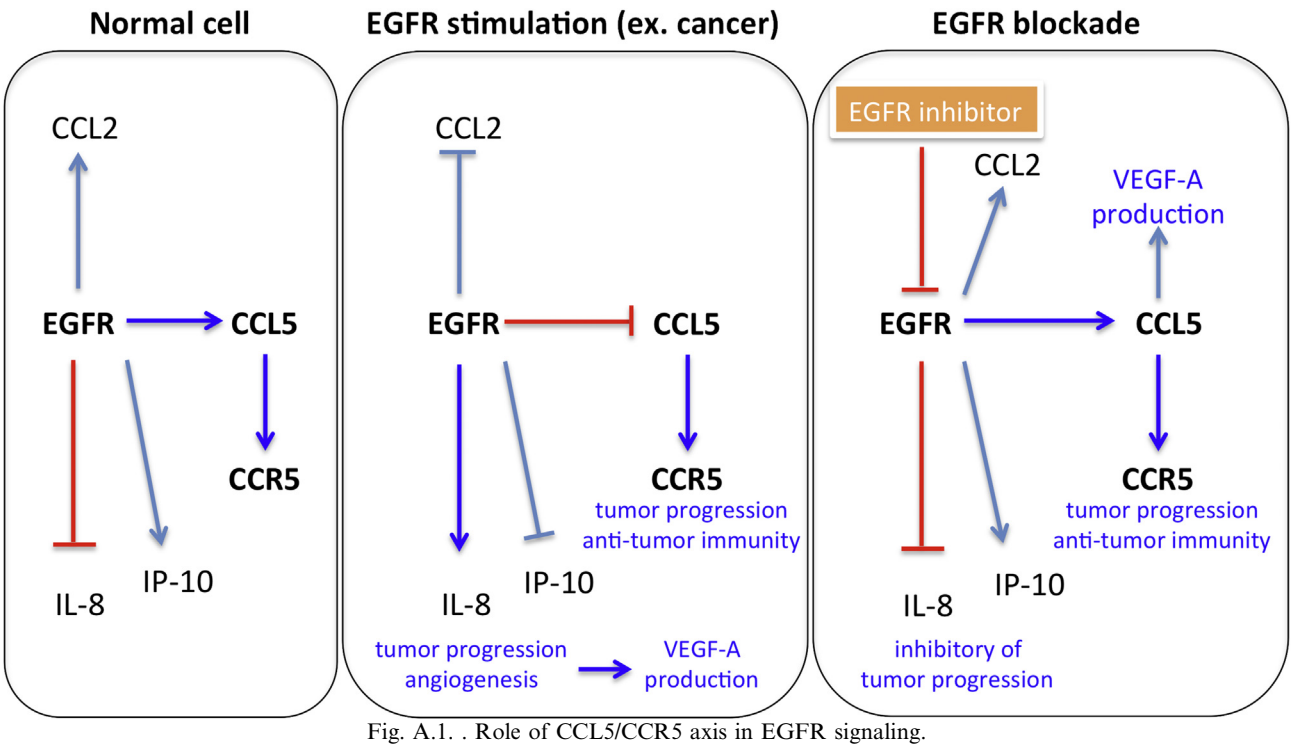

Fig. A.1. . Role of CCL5/CCR5 axis in EGFR signaling.

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