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Gene Polymorphisms in the *CCL5/CCR5* Pathway as a Genetic Biomarker for Outcome and Hand–Foot Skin Reaction in Metastatic Colorectal Cancer Patients Treated With Regorafenib

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Abstract

Regorafenib confers the benefit of longer survival in metastatic colorectal cancer patients. The CCL5/CCR5 pathway modulates endothelial progenitor cell migration and vascular endothelial growth factor A production. Genetic variants of CCL4 and CCL3 may predict outcomes, and the different frequencies of CCL5 homozygote may explain ethnic differences in the development of severe hand–foot skin reactions.

Background: The C-C motif chemokine ligand 5/C-C motif chemokine receptor 5 (*CCL5/CCR5*) pathway has been shown to induce endothelial progenitor cell migration, resulting in increased vascular endothelial growth factor A expression. We hypothesized that genetic polymorphisms in the *CCL5/CCR5* pathway predict efficacy and toxicity in patients with metastatic colorectal cancer (mCRC) treated with regorafenib.

Patients and Methods: We analyzed genomic DNA extracted from 229 tumor samples from 2 different cohorts of patients who received regorafenib: an evaluation cohort of 79 Japanese

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Disclosure

The authors have stated that they have no conflict of interest.

Supplemental Data

Supplemental methods and tables accompanying this article can be found in the online version https://doi.org/10.1016/j.clcc.2018.02.010.

patients and a validation cohort of 150 Italian patients. Single nucleotide polymorphisms of *CCL5/CCR5* pathway-related genes were analyzed by PCR-based direct sequencing.

Results: *CCL4* rs1634517 and *CCL3* rs1130371 were associated with progression-free survival in the evaluation cohort (hazard ratio [HR] 1.54, $P = .043$; HR 1.48, $P = .064$), and progression-free survival (HR 1.74, $P < .001$; HR 1.66, $P = .002$) and overall survival (HR 1.65, $P = .004$; HR 1.65, $P = .004$) in the validation cohort. The allelic frequencies of *CCL5* single nucleotide polymorphisms varied between the evaluation and validation cohorts (G/G variant in rs2280789, 21.5% vs. 1.3%, $P < .001$; T/T variant in rs3817655, 22.8% vs. 2.7%, $P < .001$). In the evaluation cohort, patients with the G/G variant in rs2280789 had a higher incidence of grade 3+ hand–foot skin reaction compared to any A allele (53% vs. 27%, $P = .078$), and similarly to the T/T variant in rs3817655 compared to any A allele (56% vs. 26%, $P = .026$).

Conclusion: Genetic variants in the *CCL5/CCR5* pathway may serve as prognostic markers and may predict severe hand–foot skin reaction in mCRC patients receiving regorafenib therapy.

Keywords

CCL5/CCR5 signaling; Colorectal cancer; Ethnic difference; Hand-foot skin reaction; Regorafenib

Introduction

Regorafenib, an oral multikinase inhibitor, confers the benefit of longer survival to patients with refractory metastatic colorectal cancer (mCRC).[1,2] Tumor mutation status, plasma DNA concentration, and plasma protein concentration, including its target protein kinases, have been examined by a retrospective exploration of the CORRECT study to identify predictive markers of this agent, while real-time circulating DNA analysis has shown potential prognostic markers for clinical outcomes.[3] However, no validated predictive markers of efficacy and/or toxicity have been identified. Hand–foot skin reaction (HFSR) is a well-known toxicity of regorafenib that obliges patients to interrupt treatment, and an ethnic difference in the frequency of HFSR has been reported between Japanese and non-Japanese patients in the CORRECT study.[4]

A recent study that investigated whether serum cytokine levels are associated with clinical outcomes in mCRC patients receiving regorafenib reported that baseline serum C-C motif chemokine ligand 5 (*CCL5*) levels and decrease of serum vascular endothelial growth factor (VEGF) A levels after start of treatment predicted the efficacy of regorafenib in refractory mCRC. Furthermore, low *CCL5* levels were associated with the onset of HFSR.[5] C-C motif chemokine receptor 5 (*CCR5*) is a receptor of *CCL5*, and *CCL5* can promote endothelial progenitor cell (EPC) migration in a *CCR5*-dependent manner. The *CCL5/CCR5* pathway is involved in VEGF-A production via EPC migration.[6] *CCL5* is characterized as late expression after T-cell activation, and it localizes with tumor-infiltrating leukocytes.[7] It is also known as regulated on activation, normal T-cell expressed and secreted (RANTES). Krüppel-like transcription factor (KLF) 13 is a transcription factor that regulates RANTES expression in T lymphocytes; it is known as RANTES factor of late activated T lymphocytes 1 (RFLAT-1).[8] Other *CCR5* ligands–C-C motif chemokine ligand-3 (*CCL3*) and −4 (*CCL4*)—also participate in EPC migration via binding to CCR5; however, a recent in vitro study

showed that *CCL5* is the most potent chemoattractant of EPCs.[9] The *CCL5/CCR5* signaling pathway positively activates protein kinase Cδ (PKCδ), c-Src, and hypoxia-inducible factor 1α (HIF1A) in activating VEGF-A expression (Figure 1).[6]

We therefore tested whether genetic polymorphisms in the *CCL5/CCR5* pathway are associated with clinical outcomes and toxicity, particularly HFSR, in patients with refractory mCRC treated with regorafenib.

Patients and Methods

Study Design and Patients

This study investigated 2 independent cohorts composed of patients with refractory, histologically confirmed mCRC: an evaluation cohort of 79 patients treated with regorafenib at the Cancer Institute Hospital in Japan between May 2013 and December 2015, and a validation cohort of 150 patients treated with regorafenib at Azienda Ospedaliero–Universitaria Pisana (Pisa, Italy) and Istituto Oncologico Veneto (Padua, Italy) between August 2010 and November 2015. All patients met the eligibility criteria: history of standard chemotherapy including 5-fluorouracil, oxaliplatin, irinotecan, bevacizumab, and cetuximab or panitumumab for *KRAS* or *RAS* wild type; measurable or evaluable disease according to the Response Evaluation Criteria in Solid Tumors v1.1; and signed informed consent. Adverse events were graded according to the Common Terminology Criteria for Adverse Events, version 4.0.

In the evaluation and validation cohorts, patients received 160 mg regorafenib (Bayer, Leverkusen, Germany) once daily from day 1 to day 21 every 4 weeks. Doses were adjusted on the basis of adverse events at a physician's discretion, following the manufacturer's recommendations. We were fully compliant with the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) guidelines. The analyses were approved by the institutional review board of each institute, and they were conducted at the University of Southern California/Norris Comprehensive Cancer Center and in accordance with the Declaration of Helsinki and good clinical practice guidelines.

Selection of Candidate Single Nucleotide Polymorphisms

The 9 candidate single nucleotide polymorphisms (SNPs) in this study inhabited 7 genes—*CCL3, CCL4, CCL5, CCR5, PRKCD, KLF13*, and *HIF1A*—and were selected on the basis of the following criteria: (1) SNP with biological significance according to published literature review; (2) tagging SNPs selected using the HapMap genotype data with r^2 threshold = 0.8 (https://snpinfo.niehs.nih.gov/snpinfo/snptag.html); or (3) minor allele frequency with a cutoff of ≥ 10% in both whites and East Asians (in the Ensembl Genome Browser, http://uswest.ensembl.org/index.html). Functional significance was predicted on the basis of the Functional Single Nucleotide Polymorphism database (http://compbio.cs.queensu.ca/F-SNP/) (Supplemental Table 1 in the online version). Details of DNA extraction and genotyping are provided in the Supplemental Methods in the online version.

Analysis of Serum VEGF-A and CCL5 Levels

Blood samples were obtained from 57 Japanese patients enrolled onto the evaluation cohort, at baseline before the first dose of regorafenib, and at day 21 in the first cycle (Supplemental Methods in the online version).

Statistical Analysis

The primary end point of the current study was progression-free survival (PFS), and the secondary end points were overall survival (OS) and disease control rate. All analyses were performed by SAS 9.4 (SAS Institute, Cary, NC). All tests were 2 sided at a significance level of .05. *P* values were not adjusted for multiple testing (Supplemental Methods in the online version).

Results

Patient and Tumor Baseline Characteristics

In the evaluation cohort, the median follow-up time was 15.3 months, and median PFS and OS were 2.0 and 8.7 months, respectively. In the validation cohort, the median follow-up time was 36.4 months, and median PFS and OS were 2.1 and 6.0 months, respectively. The baseline characteristics of the evaluation and validation are summarized in Supplemental Table 2 in the online version. The associations between baseline characteristics and clinical outcomes are summarized in Supplemental Tables 3 and 4 in the online version for evaluation and validation, respectively. All candidate SNPs were within the Hardy-Weinberg equilibrium when tested using HaploView 4.2. *CCL5* rs2280789 and *CCL5* rs3817655 showed high linkage disequilibrium in both evaluation and validation cohorts (evaluation cohort: $D' = 0.97$, $r^2 = 0.92$; validation cohort: $D' = 0.97$, $r^2 = 0.73$).

Associations Between Candidate SNPs and Clinical Outcomes in Evaluation Cohort

Patients carrying the G/G variant in *CCL5* rs2280789 showed a significant benefit in OS compared to those with any A allele per the multivariable analysis (12.9 vs. 7.9 months; hazard ratio [HR] 0.45, $P = .032$). Similarly, patients carrying the T/T variant in *CCL5* rs3817655 also showed longer OS (12.9 vs. 7.9 months, HR 0.50, $P = .055$). In the univariate analysis, patients with any A allele in *CCL4* rs1634517 had significantly shorter PFS compared to those with the C/C variant (2.0 vs. 2.5 months, HR 1.54, 95% confidence interval [CI] 0.96–2.50, $P = .043$) (Figure 2A). The effect remained in the multivariable analysis ($P = .058$). Patients carrying any A allele in CCL3 rs1130371 had shorter PFS than those with the G/G variant (2.0 vs. 2.5 months, HR 1.48, 95% CI, 0.91–2.39, $P = .064$) (Figure 2B, Table 1, Supplemental Table 5 in the online version).

Association Between Candidate SNPs and Clinical Outcomes in Validation Cohort

In the univariate analysis, patients with any A allele in *CCL4* rs1634517 had significantly shorter PFS (1.8 vs. 2.3 months, HR 1.74, 95% CI, 1.24–2.45, $P < .001$) and OS (4.4 vs. 7.9 months; HR 1.65, 1.16–2.34, $P = .004$) compared to those with the C/C variant (Figure 2C and D). This remained significant in the multivariable analysis for PFS and OS (HR 1.59, $P = .012$; HR 1.46, $P = .041$, respectively). Patients carrying any A allele in *CCL3* rs1130371

had significantly shorter PFS and OS (PFS: 1.8 vs. 2.3 months, HR 1.66, 95% CI, 1.18–2.33, $P = .002$; OS: 4.4 vs. 7.9 months, HR 1.65, 95% CI, 1.16–2.34, $P = .004$) compared to those with the G/G variant (Figure 2E and F); these effects remained significant in the multivariable model (PFS: HR 1.50, $P = .027$; OS: HR 1.44, $P = .047$). Uni- and multivariate analyses using recessive models in each *CCL5* SNP were not available for analysis owing to the low frequency of the homozygote: G/G variant in rs2280789, 2 (1.3%) of 149; and T/T variant in rs3817655, 4 (2.7%) of 149 (Table 1).

Association Between Candidate SNPs and Toxicity in Both Cohorts

Grade 3 or higher adverse events were analyzed to investigate their associations with clinical outcomes and candidate SNPs. In the evaluation and validation cohorts, patients with grade 3 or higher hypertension and rash showed longer PFS and OS, respectively (Supplemental Table 6 in the online version).

Allelic distribution of SNPs was compared between the evaluation and validation cohorts. The frequency of homozygotes in *CCL5* SNPs varied between Japanese and Italian patients (G/G variant in rs2280789, 21.5% vs. 1.3%, $P < .001$; T/T variant in rs3817655, 22.8% vs. 2.7%, $P < .001$). Grade 3 or higher HFSR was more frequent in the evaluation cohort than in the validation cohort (32.9% vs. 16.0%, $P = .004$) (Figure 3 and Table 2).

Serum CCL5 and VEGF-A Levels by SNPs in Evaluation Cohort

Associations between SNPs and cytokine levels are summarized in Table 3. The *CCL5* rs2280789 G/G variant was significantly associated with lower *CCL5* levels compared to any A allele at baseline and day 21 ($P = .003$; $P = .009$). Serum VEGF-A levels at baseline appeared to be lower in the *CCL5* rs2280789 G/G variant than those in any A allele, although no statistical significance was observed; meanwhile, it was significantly lower at day 21 ($P = .024$). Similarly, the *CCL5* rs3817655 T/T variant was associated with lower serum *CCL5* levels and VEGF-A levels at baseline and day 21 compared to any A allele (*CCL5*: $P = .015$ and $P = .006$; VEGF-A: $P = .086$ and $P = .013$). In the detection of changes between baseline and day 21, increased *CCL5* levels at day 21 were highly expressed in patients with the *CCL3* rs1130371 G/G variant (63.0 vs. 34.5%, $P = .060$), *CCL4* rs1634517 C/C variant (61.3 vs. 32.0%, $P = .035$), and the *CCR5* rs1799988 T/T variant (70.6 vs. 38.5%, $P = .042$). However, no significant difference for serum VEGF-A levels was observed.

Discussion

Our data provide the first evidence that SNPs of genes in the *CCL5/CCR5* signaling pathway are associated with not only clinical outcomes of but also HFSR caused by regorafenib in mCRC patients.

The *CCL5/CCR5* axis is involved in the immune microenvironment and is exploited for network-enabling tumor progression.[10] *CCL5* is expressed and localized within $CD8^+$ T cells and CXCL10 in tumor cells and macrophages within the invasive margin. *CCL3* and *CCL4*, macrophage inflammatory protein 1 proteins, are produced particularly by macrophages, dendritic cells, and lymphocytes activating *CCR5* downstream. RNA

expression analysis in colorectal cancer showed that *CCL4* was the most strongly expressed in cancer tissues compared to those expressed in nonneoplastic mucosal tissues. *CCL3* was also highly expressed in cancer tissue. In contrast, *CCL5* was widely expressed not only in cancer tissue but also in nonneoplastic mucosal tissues.[11]

Our approach was based on preliminary data obtained from a previous translational study that identified both low serum *CCL5* levels at baseline and decreased serum VEGF-A levels under treatment with regorafenib,[5] indicating *CCL5* as potential regulator of VEGF-A production. Recent studies demonstrated that both *CCL5* and *CCR5* are the key players in activating the signaling.[6,12] The clinical significance of gene polymorphisms in the *CCL5/CCR5* signaling pathway in carcinogenesis and their predictive and prognostic value with regard to chemotherapeutic agents remains unclear. In our study, patients with the homozygous G/G variant in *CCL5* rs2280789 or T/T variant in *CCL5* rs3817655 had a trend toward longer OS in the evaluation cohorts. However, these findings were not confirmed in the validation cohort owing to the quite low frequency of these homozygotes in the validation cohort compared to those in the evaluation cohort (approximately 1% vs. 10%). Intriguingly, SNPs of other *CCR5* ligands, *CCL4* and *CCL3*, were associated with PFS and OS in both evaluation and validation cohorts. In addition, allelic distributions of these SNPs were similar between the evaluation and validation cohorts, unlike *CCL5*. Our data are also consistent with findings that mRNA expressions of *CCL4* and *CCL3* were more specific in cancer tissue than in normal tissue, while *CCL5* expression was not limited to cancer tissue. [11] In addition, an in vivo study revealed that only *CCL5* could induce EPC migration in a dose-dependent manner at a wound site with *CCL3, CCL4, CCL5*, and *CCR5* expression, while *CCL3* and *CCL4* lacked this activity.[9]

Regarding the genetic functionality of SNPs, An et al[13] demonstrated that transcriptional regulation of *CCL5* was primarily governed by *CCL5* rs2280789 in the promoter region, to which the G allele corresponded with a strong decrease in transcriptional activity of RANTES. In our study, *CCL5* SNPs were the only ones showing a significant relationship with *CCL5* and VEGF-A, suggesting that the homozygote might have low productivity of *CCL5* leading to lower VEGF-A production. We speculate that the demand for VEGF-A increased in response to regorafenib, which was supported by a phase I study.[14] showing that plasma VEGF-A concentration increased over 21 days of multiple doses of regorafenib followed by a decrease to baseline levels during a 7-day treatment rest. By contrast, the plasma soluble VEGF receptor (VEGFR)-2 concentration as a molecular target of regorafenib showed a dose-dependent decrease in each treatment cycle.[15] VEGF-A is known to increase vascular permeability and promote angiogenesis in tumor progression, particularly through VEGFR-2 activation.[16,17] Meanwhile, another VEGF receptor, VEGFR-1, also acts as a mediator for vascular permeability, and unique cross talk between VEGFR-1 and VEGFR-2 corresponding to vascular permeability and angiogenesis was suggested.[18] Altogether, the above assumptions may help to explain the mechanism of action of *CCL5* in VEGF-A production triggered by inhibiting VEGFR-1 and VEGFR-2 in response to regorafenib.

Another interesting result of our study is the relationship between *CCL5* SNPs and the onset of severe HFSR. Assuming that recovery from HFSR mainly depends on wound-healing

ability, the individual capacity of VEGF-A production could be a critical factor in the likelihood or severity of HFSR in addition to the pathologic findings of HFSR in patients treated with multiple kinase inhibitors such as hyperkeratosis, keratinocyte necrosis, and dermal inflammation.[19] This idea corresponds to our findings that the *CCL5* rs2280789 G/G variant and *CCL5* rs3817655 T/T variant were associated with grade 3 or higher HFSR showing lower serum *CCL5* levels compared to those with the other variants. These differences can consequently explain the ethnic difference: the high incidence of severe HFSR in the evaluation cohort of Japanese patients compared to the validation cohort of Italian patients; that is, the homozygote of the *CCL5* SNPs was extremely rare in the validation cohort compared to the evaluation cohort (1%−3% vs. 21%−23%). Considering that most of the circulating *CCL5* derives from the host and not tumors,[20] *CCL5* genotyping is suggested as a solid resource for precision medicine in managing HFSR due to regorafenib.

Our study has some limitations. It has a retrospective study design; it lacks preclinical data regarding the function of the SNPs; and all cytokine data came from a Japanese population with limited cytokine testing. In addition, other different angiogenic signaling that might affect VEGF-A production could not be excluded. Ideally, a population receiving best supportive care with refractory mCRC should be tested. Further validation research is thus warranted to confirm our findings. A strength of our study is the presence of a validation group of patients with comparable clinical characteristics receiving the same treatment. Furthermore, we first clarified the relationship between serum cytokine levels and SNPs for regorafenib on the basis of data from the previous translational study.

In conclusion, *CCL5/CCR5* signaling for VEGF-A production may affect both clinical outcome and HFSR in refractory mCRC patients receiving regorafenib. *CCL4* rs1634517 and *CCL3* rs1130371 may serve as prognostic markers, and the different percentage of homozygotes in *CCL5* SNPs lead to ethnic differences in developing severe HFSR between Italian and Japanese patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Clinical Practice Points

- Regorafenib improves survival in mCRC patients.
- *CCL5/CCR5* signaling pathway modulates VEGF-A production.
- Genetic variants of *CCL4* and *CCL3* are associated with clinical outcomes.
- *CCL5* homozygote is associated with severe HFSR.
- Frequencies of *CCL5* homozygote accounts for the ethnic differences of severe HFSR.

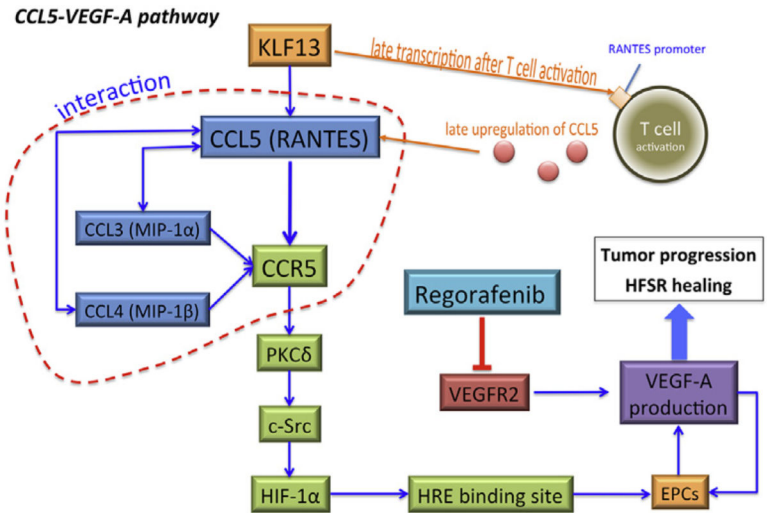

Figure 1. Illustration of *CCL5-CCR5* Signaling Pathway for VEGF-A production in Regorafenib Treatment

Abbreviations: EPC = endothelial progenitor cell; HFSR = hand–foot skin reaction; HRE = hypoxia-response element; MIP-1 = macrophage inflammatory protein 1; RANTES = regulated on activation, normal T-cell expressed and secreted; VEGF-A = vascular endothelial growth factor A.

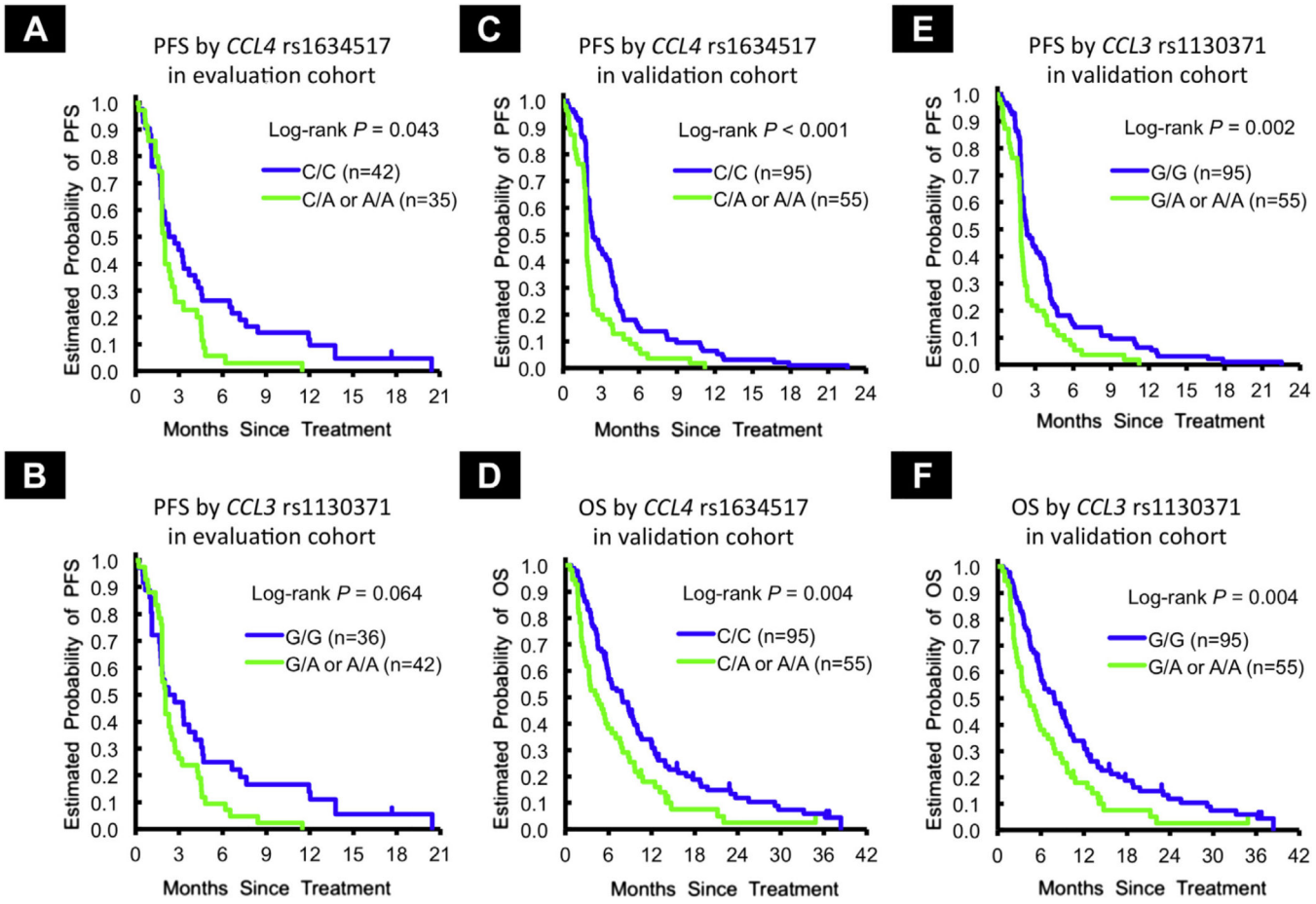

Figure 2.
PFS and OS in Evaluation and Validation Cohorts

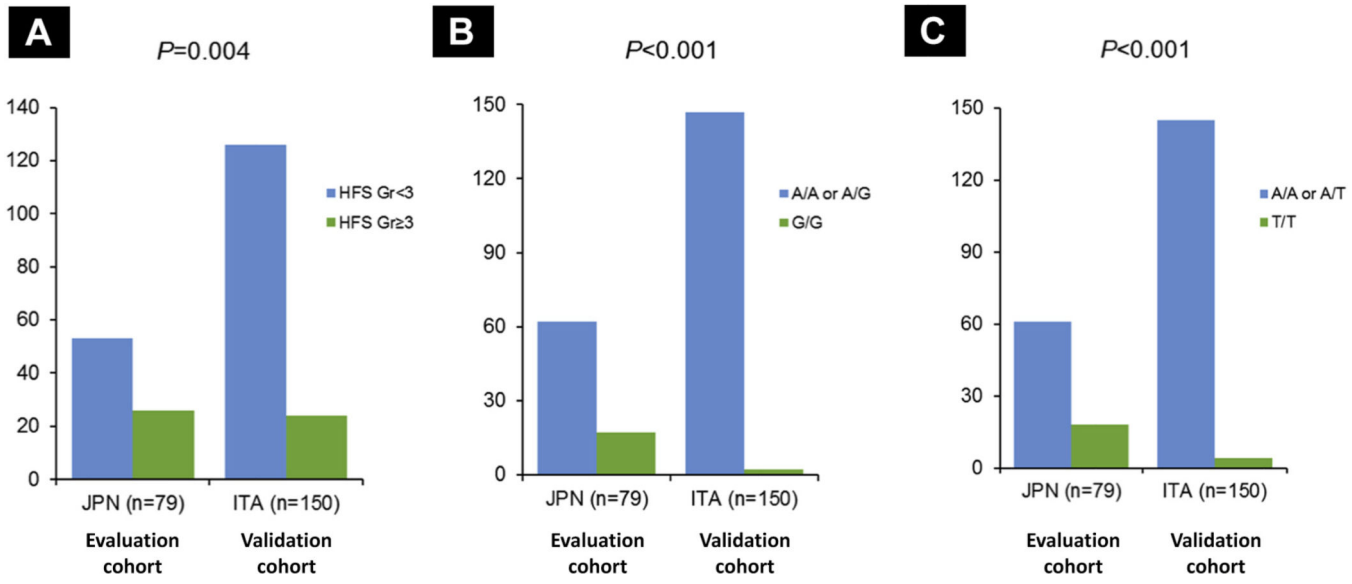

Figure 3. Different in Frequency of Grade 3 + HFSR (A), and Allelic Distribution of *CCL5* rs2280789 (B) and *CCL5* rs3817655 (C) in Evaluation and Validation Cohorts

Abbreviations: ITA = Italian; JPN = Japanese.

Table 1

Association Between Gene Polymorphism and Clinical Outcome

	N	Disease Control			Progression-Free Survival					Overall Survival				
		PR + SD	PD	*P* value*	Median (95% CI), mo	Univariate HR (95% CI)†	*P* value*	Multivariable HR (95% CI)‡	*P* value*	Median (95% CI), mo	Univariate HR (95% CI)†	*P* value*	Multivariable HR (95% CI)‡	*P* value*
CCL5 rs2280789				.42			.56		.21			.36		**.039**
A/A	27	13 (59%)	9 (41%)		2.3 (1.8, 3.3)	1 (Reference)		1 (Reference)		7.9 (4.0, 29.9)	1 (Reference)		1 (Reference)	
A/G	35	12 (46%)	14 (54%)		2.0 (1.5, 2.5)	1.01 (0.61, 1.69)		1.20 (0.72, 2.02)		8.1 (4.6, 12.6)	1.19 (0.64, 2.22)		1.53 (0.81, 2.91)	
G/G	17	6 (38%)	10 (63%)		2.0 (1.7, 4.7)	0.76 (0.41, 1.43)		0.68 (0.36, 1.30)		12.9 (5.0, 27.7)	0.71 (0.32, 1.57)		0.56 (0.25, 1.27)	
				.39			.28		.11			.19		**.032**
Any A	62	25 (52%)	23 (48%)		2.0 (1.8, 2.7)	1 (Reference)		1 (Reference)		7.9 (5.8, 11.8)	1 (Reference)		1 (Reference)	
G/G	17	6 (38%)	10 (63%)		2.0 (1.7, 4.7)	0.76 (0.43, 1.32)		0.62 (0.35, 1.11)		12.9 (5.0, 27.7)	0.64 (0.32, 1.28)		0.45 (0.21, 0.93)	
CCL5 rs3817655				.74			.60		.40			.52		.12
A/A	27	12 (55%)	10 (45%)		2.0 (1.8, 3.2)	1 (Reference)		1 (Reference)		7.1 (4.0, 29.9)	1 (Reference)		1 (Reference)	
A/T	34	12 (48%)	13 (52%)		2.0 (1.6, 3.7)	0.87 (0.52, 1.46)		0.98 (0.58, 1.67)		8.7 (4.6, 12.6)	1.00 (0.54, 1.86)		1.28 (0.67, 2.44)	
T/T	18	7 (41%)	10 (59%)		1.9 (1.7, 4.5)	0.75 (0.40, 1.38)		0.67 (0.35, 1.26)		12.9 (5.0, 27.7)	0.68 (0.32, 1.47)		0.57 (0.26, 1.24)	
				.58			.40		.18			.25		.055
Any A	61	24 (51%)	23 (49%)		2.0 (1.8, 2.7)	1 (Reference)		1 (Reference)		7.9 (5.8, 11.8)	1 (Reference)		1 (Reference)	
T/T	18	7 (41%)	10 (59%)		1.9 (1.7, 4.5)	0.81 (0.47, 1.39)		0.67 (0.38, 1.19)		12.9 (5.0, 27.7)	0.68 (0.35, 1.34)		0.50 (0.25, 1.02)	
CCR5 rs1799988				**.019**			.25		.50			.43		.55
T/T	25	5 (25%)	15 (75%)		1.8 (1.1, 2.3)	1 (Reference)		1 (Reference)		6.3 (4.0, 27.2)	1 (Reference)		1 (Reference)	

Clin Colorectal Cancer. Author manuscript; available in PMC 2020 September 16.

	N	Disease Control			Progression-Free Survival					Overall Survival				
		PR + SD	PD	*P* value*	Median (95% CI), mo	Univariate HR (95% CI)†	*P* value*	Multivariable HR (95% CI)‡	*P* value*	Median (95% CI), mo	Univariate HR (95% CI)†	*P* value*	Multivariable HR (95% CI)‡	*P* value*
T/C	29	12 (50%)	12 (50%)		2.3 (1.8, 3.3)	0.65 (0.37, 1.14)		0.71 (0.40, 1.26)		9.6 (5.0, 13.6)	0.88 (0.45, 1.70)		0.88 (0.45, 1.73)	
C/C	24	14 (70%)	6 (30%)		2.7 (1.6, 4.5)	0.74 (0.42, 1.30)		0.80 (0.45, 1.42)		12.6 (6.5, 15.5)	0.64 (0.32, 1.30)		0.68 (0.33, 1.39)	
				.016			.11		.26			.35		.43
T/T	25	5 (25%)	15 (75%)		1.8 (1.1, 2.3)	1 (Reference)		1 (Reference)		6.3 (4.0, 27.2)	1 (Reference)		1 (Reference)	
Any C	53	26 (59%)	18 (41%)		2.5 (1.8, 3.3)	0.69 (0.42, 1.12)		0.75 (0.46, 1.23)		10.3 (6.7, 12.9)	0.76 (0.42, 1.38)		0.78 (0.42, 1.44)	
				.031			.81		.86			.22		.30
Any T	54	17 (39%)	27 (61%)		2.0 (1.8, 2.3)	1 (Reference)		1 (Reference)		7.8 (5.0, 10.8)	1 (Reference)		1 (Reference)	
C/C	24	14 (70%)	6 (30%)		2.7 (1.6, 4.5)	0.94 (0.58, 1.54)		0.96 (0.58, 1.58)		12.6 (6.5, 15.5)	0.69 (0.38, 1.26)		0.73 (0.40, 1.33)	
CCL3 rs1130371				.31			.064		.41			.76		.23
G/G	36	16 (57%)	12 (43%)		2.5 (1.7, 4.1)	1 (Reference)		1 (Reference)		8.1 (4.6, 12.9)	1 (Reference)		1 (Reference)	
Any A	42	15 (42%)	21 (58%)		2.0 (1.8, 2.4)	1.48 (0.91, 2.39)		1.23 (0.75, 2.01)		9.6 (6.1, 12.9)	1.09 (0.62, 1.90)		0.70 (0.39, 1.26)	
CCL4 rs1634517				.12			.12		.14			.13		.17
C/C	42	20 (57%)	15 (43%)		2.5 (1.7, 3.7)	1 (Reference)		1 (Reference)		8.1 (5.1, 13.6)	1 (Reference)		1 (Reference)	
C/A	26	6 (30%)	14 (70%)		1.9 (1.6, 2.4)	1.60 (0.95, 2.69)		1.67 (1.00, 2.78)		11.8 (7.1, 15.3)	0.89 (0.48, 1.68)		0.79 (0.41, 1.50)	
A/A	9	5 (63%)	3 (38%)		2.0 (0.6, 4.5)	1.40 (0.66, 2.97)		1.35 (0.64, 2.85)		6.1 (1.5, 13.9)	1.96 (0.91, 4.20)		1.76 (0.80, 3.86)	
				.21			**.043**		.058			.71		.96
C/C	42	20 (57%)	15 (43%)		2.5 (1.7, 3.7)	1 (Reference)		1 (Reference)		8.1 (5.1, 13.6)	1 (Reference)		1 (Reference)	
Any A	35	11 (39%)	17 (61%)		2.0 (1.8, 2.4)	1.54 (0.96, 2.50)		1.58 (0.98, 2.53)		9.6 (6.1, 12.9)	1.11 (0.64, 1.94)		0.99 (0.56, 1.74)	

	N	Disease Control			Progression-Free Survival					Overall Survival				
		PR + SD	PD	*P* value*	Median (95% CI), mo	Univariate HR (95% CI)†	*P* value*	Multivariable HR (95% CI)‡	*P* value*	Median (95% CI), mo	Univariate HR (95% CI)†	*P* value*	Multivariable HR (95% CI)‡	*P* value*
KLF13 rs2241779				.62			.21		**.019**			.57		.20
C/C	30	14 (56%)	11 (44%)		3.0 (1.8, 4.6)	1 (Reference)		1 (Reference)		10.3 (6.1, 15.5)	1 (Reference)		1 (Reference)	
C/A	33	12 (46%)	14 (54%)		1.8 (1.5, 2.3)	1.48 (0.89, 2.45)		2.13 (1.24, 3.65)		8.1 (5.1, 12.0)	1.37 (0.74, 2.55)		1.78 (0.94, 3.40)	
A/A	15	5 (38%)	8 (62%)		2.0 (1.6, 2.7)	1.49 (0.79, 2.80)		1.83 (0.95, 3.50)		7.9 (3.1, 29.9)	1.32 (0.61, 2.85)		1.54 (0.71, 3.37)	
				.44			.079		**.006**			.29		.081
C/C	30	14 (56%)	11 (44%)		3.0 (1.8, 4.6)	1 (Reference)		1 (Reference)		10.3 (6.1, 15.5)	1 (Reference)		1 (Reference)	
Any A	48	17 (44%)	22 (56%)		1.9 (1.7, 2.3)	1.48 (0.93, 2.36)		2.02 (1.23, 3.33)		7.9 (5.2, 11.8)	1.35 (0.76, 2.40)		1.70 (0.94, 3.07)	
CCL5 rs2280789				.55			.27		.17			.29		.42
A/A	106	38 (37%)	64 (63%)		2.0 (1.8, 2.3)	1 (Reference)		1 (Reference)		5.9 (4.7, 7.8)	1 (Reference)		1 (Reference)	
A/G[a]	41	12 (32%)	26 (68%)		2.1 (1.9, 2.8)	0.82 (0.57, 1.19)		0.76 (0.52, 1.12)		8.0 (4.3, 10.1)	0.82 (0.56, 1.20)		0.85 (0.57, 1.26)	
G/G[a]	2	0 (0%)	2 (100%)											
CCL5 rs3817655				.42			.42		.10			.51		.34
A/A	98	35 (37%)	60 (63%)		2.0 (1.8, 2.3)	1 (Reference)		1 (Reference)		6.3 (5.0, 7.9)	1 (Reference)		1 (Reference)	
A/T[a]	47	15 (32%)	32 (68%)		2.2 (1.9, 2.5)	0.87 (0.62, 1.23)		0.74 (0.51, 1.06)		6.0 (4.3, 9.7)	0.89 (0.62, 1.27)		0.83 (0.57, 1.21)	
T/T[a]	4	0	4 (100%)											
CCR5 rs1799988				.32			.40		.076			.66		.21
T/T	52	13 (27%)	36 (73%)		2.1 (1.8, 2.3)	1 (Reference)		1 (Reference)		5.5 (4.0, 7.8)	1 (Reference)		1 (Reference)	
T/C	64	24 (39%)	37 (61%)		2.1 (1.8, 3.7)	0.78 (0.54, 1.13)		0.64 (0.43, 0.94)		8.9 (5.7, 10.5)	0.84 (0.57, 1.24)		0.70 (0.47, 1.04)	

Clin Colorectal Cancer. Author manuscript; available in PMC 2020 September 16.

	N	Disease Control			Progression-Free Survival					Overall Survival				
		PR + SD	PD	*P* value*	Median (95% CI), mo	Univariate HR (95% CI)†	*P* value*	Multivariable HR (95% CI)‡	*P* value*	Median (95% CI), mo	Univariate HR (95% CI)†	*P* value*	Multivariable HR (95% CI)‡	*P* value*
C/C	34	13 (39%)	20 (61%)		1.9 (1.8, 3.4)	0.89 (0.57, 1.37)		0.76 (0.49, 1.18)		5.6 (3.4, 8.7)	0.93 (0.59, 1.47)		0.87 (0.54, 1.40)	
				.14			.22		**.032**			.44		.12
T/T	52	13 (27%)	36 (73%)		2.1 (1.8, 2.3)	1 (Reference)		1 (Reference)		5.5 (4.0, 7.8)	1 (Reference)		1 (Reference)	
Any C	98	37 (39%)	57 (61%)		2.1 (1.8, 2.7)	0.81 (0.58, 1.15)		0.68 (0.48, 0.97)		7.1 (5.1, 9.5)	0.87 (0.61, 1.24)		0.75 (0.52, 1.08)	
CCL3 rs1130371				.10			**.002**		**.027**			**.004**		**.047**
G/G	95	37 (40%)	55 (60%)		2.3 (2.1, 3.4)	1 (Reference)		1 (Reference)		7.9 (5.9, 9.6)	1 (Reference)		1 (Reference)	
Any A	55	13 (25%)	38 (75%)		1.8 (1.7, 2.0)	1.66 (1.18, 2.33)		1.50 (1.05, 2.15)		4.4 (2.7, 6.4)	1.65 (1.16, 2.34)		1.44 (1.01, 2.07)	
CCL4 rs1634517				.077			**.003**		**.040**			**.004**		**.034**
C/C	95	37 (41%)	54 (59%)		2.3 (2.1, 3.6)	1 (Reference)		1 (Reference)		7.9 (5.9, 9.6)	1 (Reference)		1 (Reference)	
C/A	48	10 (22%)	35 (78%)		1.8 (1.7, 2.0)	1.73 (1.21, 2.48)		1.58 (1.08, 2.30)		4.9 (2.7, 7.6)	1.56 (1.08, 2.24)		1.37 (0.94, 1.99)	
A/A	7	3 (43%)	4 (57%)		1.7 (0.9, 3.1)	1.78 (0.82, 3.89)		1.72 (0.78, 3.78)		3.4 (1.0, 5.9)	2.67 (1.22, 5.86)		2.53 (1.15, 5.56)	
				.070			**<.001**		**.012**			**.004**		**.041**
C/C	95	37 (41%)	54 (59%)		2.3 (2.1, 3.6)	1 (Reference)		1 (Reference)		7.9 (5.9, 9.6)	1 (Reference)		1 (Reference)	
Any A	55	13 (25%)	39 (75%)		1.8 (1.7, 2.0)	1.74 (1.24, 2.45)		1.59 (1.11, 2.29)		4.4 (2.7, 6.4)	1.65 (1.16, 2.34)		1.46 (1.01, 2.09)	
KLF13 rs2241779				.21			.28		.17			.88		.51
C/C	33	13 (43%)	17 (57%)		2.3 (1.8, 4.1)	1 (Reference)		1 (Reference)		8.0 (3.6, 10.0)	1 (Reference)		1 (Reference)	
A/C	72	19 (28%)	50 (72%)		1.9 (1.8, 2.1)	1.38 (0.91, 2.09)		1.37 (0.90, 2.09)		5.9 (4.5, 7.8)	1.06 (0.69, 1.63)		1.05 (0.68, 1.62)	
A/A	44	17 (40%)	26 (60%)		2.3 (1.9, 2.8)	1.15 (0.73, 1.82)		0.99 (0.62,1.58)		5.7 (4.4, 9.5)	0.96 (0.60, 1.53)		0.83 (0.51, 1.34)	

	N	Disease Control			Progression-Free Survival					Overall Survival				
		PR + SD	PD	*P* value*	Median (95% CI), mo	Univariate HR (95% CI)†	*P* value*	Multivariable HR (95% CI)‡	*P* value*	Median (95% CI), mo	Univariate HR (95% CI)†	*P* value*	Multivariable HR (95% CI)‡	*P* value*
				.28			.21		.38			.93		.83
C/C	33	13 (43%)	17 (57%)		2.3 (1.8, 4.1)	1 (Reference)		1 (Reference)		8.0 (3.6, 10.0)	1 (Reference)		1 (Reference)	
Any A	116	36 (32%)	76 (68%)		2.0 (1.8, 2.3)	1.28 (0.86, 1.90)		1.20 (0.80, 1.78)		5.9 (4.7, 7.8)	1.02 (0.68, 1.52)		0.96 (0.64, 1.44)	

Abbreviations: PD = progressive disease; PR = partial response; SD = stable disease.

P values<.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 liver metastasis and LN metastasis in the evaluation cohort; time to REGO started (<18 vs ≥18 months), ECOG performance status (0 vs 1 or 2), primary tumor resection (yes vs no), and Kohne score (low-intermediate vs high) in the validation cohort.

*[a]*Grouped together for estimate of HR.

Clin Colorectal Cancer. Author manuscript; available in PMC 2020 September 16.

Table 2

Association Between SNPs and Toxicities

	Evaluation Cohort				Validation Cohort			
SNP	**N**	**Grade 3 or Higher**	**Grade 2 or Lower**	***P***	**N**	**Grade 3 or Higher**	**Grade 2 or Lower**	***P***
Hand–Foot Skin Reaction								
CCL5 rs2280789				.16				.24
A/A	27	8 (30%)	19 (70%)		106	15 (14%)	91 (86%)	
A/G	35	9 (26%)	26 (74%)		41	8 (20%)	33 (80%)	
G/G	17	9 (53%)	8 (47%)		2	1 (50%)	1 (50%)	
				.078				.30
Any A	62	17 (27%)	45 (73%)		147	23 (16%)	124 (84%)	
G/G	17	9 (53%)	8 (47%)		2	1 (50%)	1 (50%)	
CCL5 rs3817655				.084				.67
A/A	27	7 (26%)	20 (74%)		98	15 (15%)	83 (85%)	
A/T	34	9 (26%)	25 (74%)		47	8 (17%)	39 (83%)	
T/T	18	10 (56%)	8 (44%)		4	1 (25%)	3 (75%)	
				.026[a]				.51
Any A	61	16 (26%)	45 (74%)		145	23 (16%)	122 (84%)	
T/T	18	10 (56%)	8 (44%)		4	1 (25%)	3 (75%)	
Hypertension								
CCL5 rs2280789				.037[a]				.81
A/A	27	0	27 (100%)		106	25 (24%)	81 (76%)	
A/G	35	6 (17%)	29 (83%)		41	11 (27%)	30 (73%)	
G/G	17	1 (6%)	16 (94%)		2	0	2 (100%)	
				.089				.83
A/A	27	0	27 (100%)		106	25 (24%)	81 (76%)	
Any G	52	7 (13%)	45 (87%)		43	11 (26%)	32 (74%)	
CCL5 rs3817655				.040[a]				.75
A/A	27	0	27 (100%)		98	24 (24%)	74 (76%)	

SNP	Evaluation Cohort				Validation Cohort			
	N	Grade 3 or Higher	Grade 2 or Lower	*P*	N	Grade 3 or Higher	Grade 2 or Lower	*P*
A/T	34	6 (18%)	28 (82%)		47	12 (26%)	35 (74%)	
T/T	18	1 (6%)	17 (94%)		4	0	4 (100%)	
				.089				1.00
A/A	27	0	27 (100%)		98	24 (24%)	74 (76%)	
Any T	52	7 (13%)	45 (87%)		51	12 (24%)	39 (76%)	
CCR5 rs1799988				.026[a]				.37
Any T	54	2 (4%)	52 (96%)		116	30 (26%)	86 (74%)	
C/C	24	5 (21%)	19 (79%)		34	6 (18%)	28 (82%)	
Diarrhea								
CCL5 rs2280789				.52				.034[a]
A/A	27	0	27 (100%)		106	11 (10%)	95 (90%)	
Any G	52	3 (6%)	49 (94%)		43	0	43 (100%)	
Rash								
KLF13 rs2241779				.50				.010[a]
C/C	30	5 (17%)	25 (83%)		33	10 (30%)	23 (70%)	
Any A	48	5 (10%)	43 (90%)		116	12 (10%)	104 (90%)	
AST/ALT								
CCL3 rs1130371				.65				.004[a]
G/G	36	2 (6%)	34 (94%)		95	7 (7%)	88 (93%)	
G/A	39	1 (3%)	38 (97%)		52	1 (2%)	51 (98%)	
A/A	3	0	3 (100%)		3	2 (67%)	1 (33%)	
				.59				.75
G/G	36	2 (6%)	34 (94%)		95	7 (7%)	88 (93%)	
Any A	42	1 (2%)	41 (98%)		55	3 (5%)	52 (95%)	
CCL4 rs1634517				.22				.052
C/C	42	2 (5%)	40 (95%)		95	7 (7%)	88 (93%)	
C/A	26	0	26 (100%)		48	1 (2%)	47 (98%)	

Clin Colorectal Cancer. Author manuscript; available in PMC 2020 September 16.

SNP	Evaluation Cohort				Validation Cohort			
	N	Grade 3 or Higher	Grade 2 or Lower	*P*	N	Grade 3 or Higher	Grade 2 or Lower	*P*
A/A	9	1 (11%)	8 (89%)		7	2 (29%)	5 (71%)	
				1.00				.75
C/C	42	2 (5%)	40 (95%)		95	7 (7%)	88 (93%)	
Any A	35	1 (3%)	34 (97%)		55	3 (5%)	52 (95%)	

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; SNP = single nucleotide polymorphism.

[a]Statistically significant ($P < .05$) by Fisher's exact test.

Table 3

Association Between Serum Cytokine Levels and SNPs in Evaluation Cohort

SNP	N	Mean Serum *CCL5* Levels (pg/mL)				Mean Serum VEGF-A Levels (pg/mL)			
		Baseline	Day 21	Quantitative Change	Increase (%)	Baseline	Day 21	Quantitative Change	Increase (%)
***CCL5* rs2280789**									
A/A	20	67,125.2	66,218.1	−1167.7	47.4	404.0	621.0	205.2	68.4
A/G	22	63,290.4	64,017.6	727.2	50.0	369.4	509.0	139.6	54.5
G/G	15	44,844.5	43,344.6	−1499.9	46.7	251.4	324.1	72.7	66.7
P		.010[a]	.008[a]	.97	1.00	.14	.062	.34	.65
Any A	42	65,116.5	65,037.4	−150.9	48.8	385.8	560.9	170.0	61.0
G/G	15	44,844.5	43,344.6	−1499.9	46.7	251.4	324.1	72.7	66.7
P		.003[a]	.009[a]	.82	1.00	.13	.024[a]	.23	.76
***CCL5* rs3817655**									
A/A	20	67,125.2	66,218.1	−1167.7	47.4	404.0	621.0	205.2	68.4
A/T	21	61,796.6	64,922.4	3125.8	52.4	381.9	525.8	143.9	52.4
T/T	16	47,958.0	43,449.1	−4508.8	43.8	242.3	313.5	71.2	68.8
P		.039[a]	.005[a]	.68	.94	.45	.041[a]	.32	.58
Any A	41	64,396.0	65,537.9	1086.4	50.0	392.7	571.0	173.0	60.0
T/T	16	47,958.0	43,449.1	−4508.8	43.8	242.3	313.5	71.2	68.8
P		.015[a]	.006[a]	.39	.77	.086	.013[a]	.21	.76
***CCL3* rs1130371**									
G/G	28	58,307.0	59,695.6	1531.8	63.0	325.3	423.0	92.3	66.7
G/A	27	60,243.5	57,254.6	−2988.9	33.3	369.6	539.1	169.5	55.6
A/A	2	74,195.5	79,523.5	5328.0	50.0	444.4	940.8	496.5	100
P		.65	.56	.81	.078	.87	.64	.29	.55
G/G	28	58,307.0	59,695.6	1531.8	63.0	325.3	423.0	92.3	66.7
Any A	29	61,205.7	58,790.3	−2415.3	34.5	374.8	566.8	192.0	58.6
P		.64	.91	.60	.060	.65	.23	.32	.59

SNP	N	Mean Serum *CCL5* Levels (pg/mL)				Mean Serum VEGF-A Levels (pg/mL)			
		Baseline	Day 21	Quantitative Change	Increase (%)	Baseline	Day 21	Quantitative Change	Increase (%)
Any G	55	59,257.6	58,475.1	−728.6	48.1	347.0	481.0	130.9	61.1
A/A	2	74,195.5	79,523.5	5328.0	50	444.4	940.8	496.5	100
P		.38	.30	.77	1.00	.74	.70	.17	.52
***CCL4* rs1634517**									
C/C	32	56,542.2	60,138.3	3777.7	61.3	318.8	415.0	91.7	61.3
C/A	17	58,021.9	56,411.3	−1610.6	41.2	392.7	623.8	231.1	70.6
A/A	8	76,479.8	61,677.9	−14,801.9	12.5	387.4	548.3	160.9	50.0
P		.085	.88	.24	.036[a]	.80	.29	.46	.64
C/C	32	56,542.2	60,138.3	3777.7	61.3	318.8	415.0	91.7	61.3
Any A	25	63,928.4	58,096.6	−5831.8	32.0	391.0	599.7	208.6	64.0
P		.24	.79	.20	.035[a]	.51	.14	.24	1.00
Any C	49	57,055.6	58,818.3	1869.4	54.2	344.4	489.0	141.1	64.6
A/A	8	76,479.8	61,677.9	−14,801.9	12.5	387.4	548.3	160.9	50.0
P		.026[a]	.79	.12	.052	.78	.73	.89	.46
***CCR5* rs1799988**									
T/T	17	55,005.2	62,594.8	7589.5	70.6	288.4	436.2	147.8	70.6
T/C	21	60,885.6	56,854.4	−3966.9	45	386.8	608.0	210.8	65.0
C/C	19	62,835.4	58,710.6	−4124.8	31.6	365.7	435.9	70.2	52.6
P		.59	.82	.36	.071	.75	.39	.48	.54
T/T	17	55,005.2	62,594.8	7589.5	70.6	288.4	436.2	147.8	70.6
Any C	40	61,811.8	57,758.7	−4043.8	38.5	376.8	524.2	142.3	59.0
P		.31	.56	.15	.042[a]	.45	.50	.96	.55
Any T	38	58,254.9	59,491.9	1342.8	56.8	342.8147	529.0511	181.8	67.6
C/C	19	62,835.4	58,710.6	−4124.8	31.6	365.7442	435.9084	70.2	52.6
P		.49	.92	.49	.095	.84	.40	.29	.38

Abbreviations: SNP = single nucleotide polymorphism; VEGF-A = vascular endothelial growth factor A.

[a]Statistically significant ($P < .05$). *P* values were calculated by Student's unpaired *t* test and 1-way ANOVA for means of continuous measurements and Fisher's exact test for change patterns in each cytokines.