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# Prognostic Effect of Adenosine-related Genetic Variants in Metastatic Colorectal Cancer Treated With Bevacizumab-based Chemotherapy

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## Abstract

**Adenosine has an immunosuppressive and angiogenic modulatory role in the tumor microenvironment. The present study revealed that *CD39 rs11188513*, a single nucleotide polymorphism in the adenosine pathway, affected the clinical outcomes of 451 patients with metastatic colorectal cancer from 2 phase III clinical trials treated with FOLFIRI (5-fluorouracil, leucovorin, oxaliplatin, irinotecan) plus bevacizumab.**

**Background:** Adenosine has an immunosuppressive and angiogenic modulation of the tumor microenvironment. The present study explored the efficacy of single nucleotide polymorphisms (SNPs) in adenosine-related molecules for patients with metastatic colorectal cancer treated with bevacizumab-based chemotherapy. **Patients and Methods:** We analyzed genomic DNA extracted from 451 samples from 3 independent cohorts: a discovery cohort of 107 patients treated with FOLFIRI (5-fluorouracil, leucovorin, oxaliplatin, irinotecan) plus bevacizumab in FIRE-3 (ClinicalTrials.gov identifier, NCT00433927); a validation cohort of 215 patients with FOLFIRI plus bevacizumab in TRIBE (ClinicalTrials.gov identifier, NCT00719797); and a control cohort of 129 patients treated with FOLFIRI plus cetuximab in FIRE-3. The relationship between the selected SNPs and clinical outcomes was analyzed. **Results:** In the discovery cohort, patients with any C allele in *CD39 rs11188513* had significantly shorter median progression-free survival compared with those with the T/T variant (11.3 vs. 13.1 months; hazard ratio [HR], 1.70; 95% confidence interval [CI], 1.04-2.77; $P = .022$) on univariate analysis. Also, their overall survival (OS) was shorter (27.4 vs. 49.9 months; HR, 2.10; 95% CI, 1.07-4.10; $P = .031$) on univariate and multivariable analyses. The significant association between *CD39 rs11188513* and OS was confirmed in the validation cohort (25.8 vs. 31.6 months; HR, 1.53; 95% CI, 1.09-2.15; $P = .013$). *CD73 rs2229523* and *A2BR rs2015353* in the discovery cohort and *CD39 rs2226163* in the validation cohort showed significant correlations with OS on univariate and multivariable analyses. None of SNPs were significant in the cetuximab control cohort. **Conclusion:** Selected SNPs in the adenosine pathway could affect the clinical outcomes of patients with metastatic colorectal cancer treated with FOLFIRI plus bevacizumab.

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**Keywords:** A2AR, CD39, CD73, mCRC, SNP

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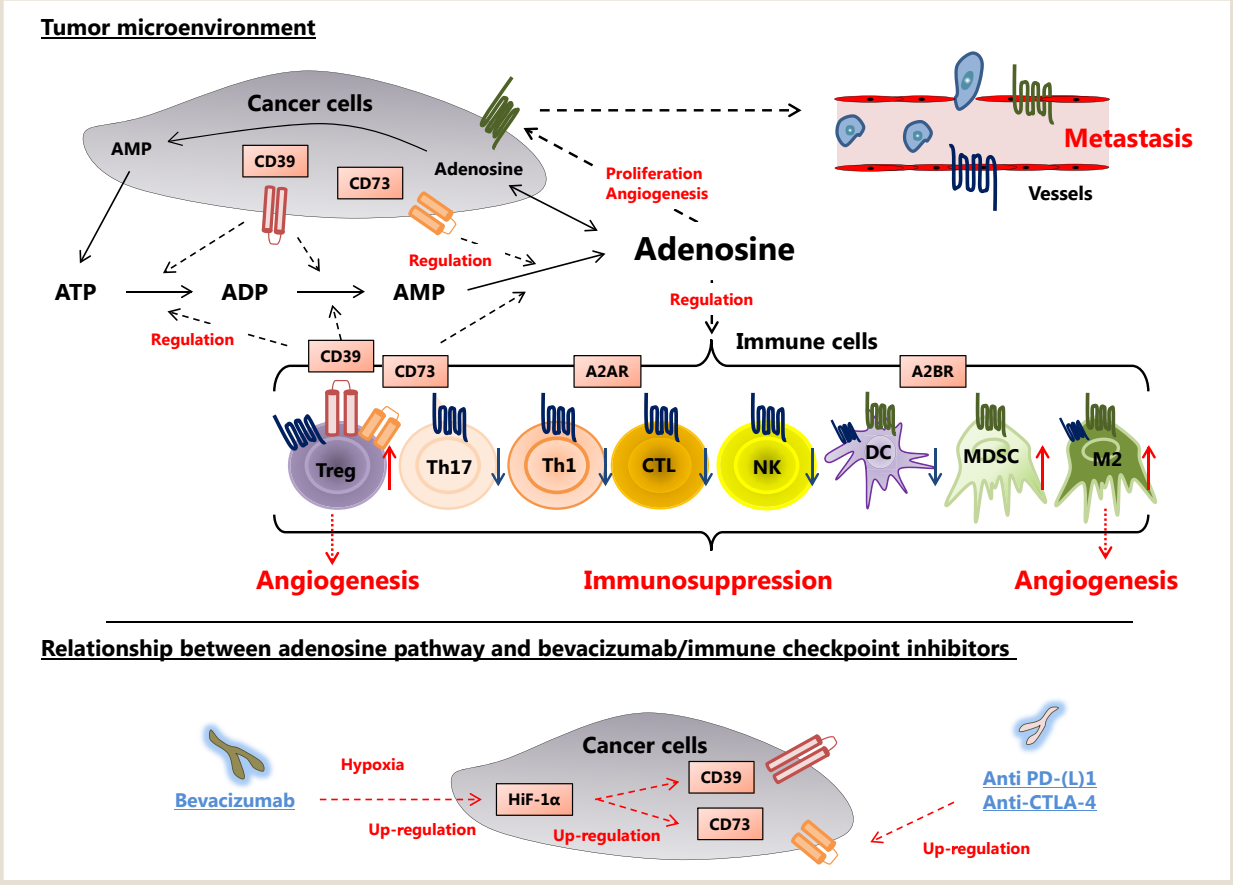
## Introduction

Adenosine has a potent immunosuppressive ability and is autonomously produced from adenosine triphosphate through adenosine diphosphate via CD39 and CD73, 2 ectonucleotidases, expressed mainly in the surface membrane of cancer cells, B cells or regulatory T cells (Tregs).[1-3] Although extracellular adenosine triphosphate enhances immune cell chemotaxis and activities, extracellular adenosine rapidly acts on immune cells and leads to immunosuppression in the tumor microenvironment.[4] Extracellular adenosine has 4 receptors (A1R, A2AR, A2BR, and A3R).[5,6] Of these, A2AR is predominantly present on the surface of the lymphocytes, and A2BR is mainly present in myeloid cells.[7,8] Furthermore, extracellular adenosine also acts on cancer cells through A2BR and stimulates tumor growth and metastasis[9] (Figure 1). Consistent with these functions, previous reports have shown that the molecules of adenosine pathway can be prognostic factors and therapeutic targets in various cancers.[10-12] Blockade of the adenosine pathway also increases the therapeutic efficacy of anti–programmed cell death 1 (PD-1) or anti–cytotoxic T-lymphocyte (CTL)–associated protein 4 (CTLA-4) in preclinical models, suggesting the expression of adenosine-related molecules could be a biomarker for the efficacy of checkpoint inhibitors.[13,14]

Extracellular adenosine production is strongly activated in the hypoxic state. Hypoxia-inducible factor-1α (HIF-1α) increases extracellular adenosine production through upregulation of CD39 and CD73,[15] leading to suppression of CTLs and natural killer cells[16] (Figure 1). Given the critical effects of adenosine on immune conditioning and tumor angiogenesis, it is of clinical significance to examine the association between adenosine-related molecules and the effect of anti–vascular endothelial growth factor (VEGF) therapy. Although some reports have shown that the expression of adenosine-related molecules has a strong association with survival of cancer patients,[17,18] the predictive or prognostic role of genetic changes within adenosine-related molecules remains unknown.

We hypothesized that the single nucleotide polymorphisms (SNPs) in adenosine-related molecules were associated with immune dysregulation and the efficacy of bevacizumab. Therefore, we investigated the relationships between the SNPs and clinical

**Figure 1** **Mechanism of Adenosine Pathway in Tumor Microenvironment. Adenosine Is Autonomously Produced From Adenosine Triphosphate Through Adenosine Diphosphate via CD39 and CD73 Expressed Mainly on the Surface of Cancer Cells, B Cells or Regulatory T Cells (Tregs). The Work of Extracellular Adenosine Is Mainly Divided in 2 Directions. For Immune Cells, Adenosine has a Potent Immunosuppressive Ability Through A2AR and A2BR. For Cancer Cells, Adenosine Stimulates Tumor Growth and Metastasis Through A2BR**




Abbreviations: CTL, cytotoxic lymphocyte; DC, dendritic cell; NK, natural killer; M2, M2 macrophage; MDSC, myeloid-derived suppressor cell; Th1, T helper 1; Th17, T helper 17.

outcomes in patients with metastatic colorectal cancer (mCRC) who had received bevacizumab-based chemotherapy, with cetuximab-based chemotherapy as the control. Our findings suggest that the selected SNPs in adenosine-related molecules could be biomarkers for bevacizumab-based chemotherapy and promising therapeutic targets in mCRC.

## Patients and Methods

### *Baseline Patients and Study Design*

The study subjects were 451 patients with mCRC who had undergone chemotherapy. The patients had received FOLFIRI (5-fluorouracil, leucovorin, oxaliplatin, irinotecan) plus bevacizumab or cetuximab as first-line chemotherapy in 2 prospective, randomized, open-label, phase III clinical trials: FIRE-3[19] (ClinicalTrials.gov identifier, NCT00433927) and TRIBE[20] (ClinicalTrials.gov identifier, NCT00719797). We selected the patients treated with FOLFIRI plus bevacizumab in FIRE-3 as the discovery cohort (n = 107), the patients treated with FOLFIRI plus bevacizumab in TRIBE as the validation cohort (n = 215), and the patients treated with FOLFIRI plus cetuximab in FIRE-3 as the negative control cohort ($n$ = 129). Patients without sufficient samples for analysis were excluded. The institutional review board of each participating institute approved the use of the clinical data and clinical samples for molecular analysis. The present study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines.

### *Selection of SNPs and Genotyping*

Polymorphisms underlying the adenosine-related molecules (CD39 [*ENTPD*1: *rs11188513*, *rs2226163*], CD73 [*NT5E*: *rs2229523*], A2AR [*ADORA2A*: *rs5751876*], A2BR [*ADORA2B*: *rs2015353*], and HIF-1α [*HIF1A*: *rs2057482*, *rs11549465*]), were selected. The following predefined criteria were used: (1) biological significance according to a review of the reported data; (2) a cutoff of minor allele frequency of ≥10% in whites (Ensemble Genome Browser; available at: https://www.ensembl.org/index.html); and (3) tag SNPs chosen by the HapMap genotype data with an $r^2$ threshold of 0.8 (available at: https://snpinfo.niehs.nih.gov/snpinfo/snptag.html; Supplemental Table 1; available in the online version). Genomic DNA was extracted from peripheral whole blood samples from the patients using the QIAmp Kit (Qiagen, Valencia, CA) in accordance with the manufacturer's protocol (available at: www.qiagen.com). The OncoArray was used for genotyping in the present study (Illumina, San Diego, CA).

### *Statistical Analysis*

The primary purpose of the present study was to evaluate the associations of SNPs in adenosine-related molecules with the tumor response, progression-free survival (PFS), and overall survival (OS). Patients were defined as responders when a complete or partial response was achieved and as nonresponders when stable or progressive disease was present, as defined by the Response Evaluation Criteria in Solid Tumors, version 1.1. The comparison of baseline patient characteristics between the cohorts and the correlation between SNPs and tumor response were analyzed using the $\chi^2$ test. Kaplan-Meier plots and log-rank tests were performed to evaluate the association between the candidate SNPs and clinical outcomes, PFS, and OS. The Cox proportional hazards regression model and Wald tests were used to reevaluate the independent effect between the candidate SNPs and PFS and OS. All statistical analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC). All tests were 2-sided at a significance level of $P = .05$.

## Results

### *Patient and Tumor Characteristics*

The baseline characteristics of the patients in the discovery (FIRE-3; FOLFIRI plus bevacizumab), validation (TRIBE; FOLFIRI plus bevacizumab), and control (FIRE-3; FOLFIRI plus cetuximab) cohorts are listed in Supplemental Table 2 (available in the online version). The median follow-up time was 26.7 months for the discovery cohort, 49.0 months for the validation cohort, and 29.2 months for the control cohort. The median PFS and OS were 11.6 months and 31.5 months in the discovery cohort, 9.7 months and 26.3 months in the validation cohort, and 12.8 months and 49.8 months in the control cohort, respectively. Compared with the TRIBE cohort, the patients in the FIRE-3 cohort had an older median age ($P = .006$), greater Eastern Cooperative Oncology Group (ECOG) performance status ($P < .001$), greater rates of primary tumor resection ($P < .001$), and much lower KRAS ($P < .001$) and RAS ($P < .001$) mutation rates. The control cohort had more men than the other 2 cohorts ($P = .013$).

### *Predictive and Prognostic Values of Adenosine-related SNPs in the Discovery Cohort*

The associations between the selected adenosine-related SNPs and clinical outcomes are listed in Table 1. Of the 7 candidate SNPs, *CD39 rs11188513*, *CD73 rs2229523*, and *A2BR rs2015353* were significantly associated with the clinical outcomes in the discovery cohort.

On univariate analysis, patients with mCRC and any C allele in *CD39 rs11188513* experienced significantly shorter median PFS (11.3 vs. 13.1 months; hazard ratio [HR], 1.70; 95% confidence interval [CI], 1.04-2.77; $P = .022$) and OS (27.4 vs. 49.9 months; HR, 2.19; 95% CI, 1.15-4.14; $P = .012$) than those with the T/T variant (Figure 2A). Other SNPs also resulted in significant differences in OS. Patients carrying any A allele in *CD73 rs2229523* experienced significantly longer median OS (41.9 vs. 23.7 months; HR, 0.50; 95% CI, 0.28-0.91; $P = .017$) than those with the G/G variant. Also, patients carrying the T/T variant in *A2BR rs2015353* experienced significantly longer median OS (49.9 vs. 28.1 months; HR, 0.34; 95% CI, 0.14-0.84; $P = .008$) than those with any C allele (Supplemental Figure 1; available in the online version). On multivariable analysis, *CD39 rs11188513* did not show a statistically significant difference for PFS; however, a trend was seen (HR, 1.60; 95% CI, 0.95-2.71; $P = .080$). In addition, all 3 SNPs remained significant for OS: *CD39 rs11188513* (HR, 2.10; 95% CI, 1.07-4.10; $P = .031$), *CD73 rs2229523* (HR, 0.49; 95% CI, 0.26-0.92; $P = .026$), *A2BR rs2015353* (HR, 0.24; 95% CI, 0.09-0.64; $P = .004$).

### *Confirmation of Predictive and Prognostic Effect of Adenosine-related SNPs in Validation Cohort*

Of the 3 SNPs, *CD39 rs11188513* was also significantly associated with survival in the validation cohort. In addition, *CD39*

**Table 1** Associations Between Adenosine-related SNPs and Clinical Outcomes

| SNP | Patients (n) | Tumor Response[a] | | | PFS,[b] mo | | | | | OS,[b] mo | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Yes | No | *P* Value | Median (95% CI) | HR (95% CI) | *P* Value | Adjusted HR (95% CI) | *P* Value | Median (95% CI) | HR (95% CI) | *P* Value | Adjusted HR (95% CI) | *P* Value |
| *CD39 rs11188513* | | | | | | | | | | | | | | |
| Discovery cohort (FOLFIRI, Bev) | | | | .92 | | | .022 | | .080 | | | .012 | | .031 |
| T/T | 42 | 27 (64.3) | 15 (35.7) | | 13.1 (9.9-16.9) | 1 (Ref) | | 1 (Ref) | | 49.9 (28.1-NE) | 1 (Ref) | | 1 (Ref) | |
| Any C | 63 | 38 (63.3) | 22 (36.7) | | 11.3 (9.7-12.4) | 1.70 (1.04-2.77) | | 1.60 (0.95-2.71) | | 27.4 (19.4-40.0) | 2.19 (1.15-4.14) | | 2.10 (1.07-4.10) | |
| Validation cohort (FOLFIRI, Bev) | | | | .30 | | | .10 | | .052 | | | .014 | | .013 |
| T/T | 77 | 46 (63.0) | 27 (37.0) | | 9.6 (8.8-11.7) | 1 (Ref) | | 1 (Ref) | | 31.6 (21.1-42.7) | 1 (Ref) | | 1 (Ref) | |
| Any C | 136 | 74 (55.6) | 59 (44.4) | | 9.9 (8.8-11.0) | 1.30 (0.94-1.80) | | 1.41 (1.00-1.98) | | 25.8 (21.1-28.6) | 1.50 (1.08-2.09) | | 1.53 (1.09-2.15) | |
| Control cohort (FOLFIRI, Cet) | | | | .99 | | | .66 | | .35 | | | .26 | | .12 |
| T/T | 47 | 34 (77.3) | 10 (22.7) | | 11.8 (9.0-14.1) | 1 (Ref) | | 1 (Ref) | | 37.5 (27.1-67.4) | 1 (Ref) | | 1 (Ref) | |
| Any C | 80 | 58 (77.3) | 17 (22.7) | | 13.3 (10.3-15.1) | 0.92 (0.61-1.37) | | 0.82 (0.54-1.24) | | 52.0 (42.1-NE) | 0.70 (0.37-1.32) | | 0.59 (0.31-1.14) | |
| *CD39 rs2226163* | | | | | | | | | | | | | | |
| Discovery cohort (FOLFIRI, Bev) | | | | .50 | | | .53 | | .83 | | | .21 | | .22 |
| Any A | 82 | 52 (65.8) | 27 (34.2) | | 11.3 (9.9-13.1) | 1 (Ref) | | 1 (Ref) | | 28.6 (24.7-44.3) | 1 (Ref) | | 1 (Ref) | |
| G/G | 24 | 14 (58.3) | 10 (41.7) | | 11.6 (8.6-20.5) | 0.84 (0.48-1.46) | | 0.94 (0.53-1.68) | | 49.9 (21.5-NE) | 0.62 (0.29-1.33) | | 0.61 (0.28-1.35) | |
| Validation cohort (FOLFIRI, Bev) | | | | .44 | | | .24 | | .20 | | | .023 | | .024 |
| Any A | 165 | 92 (57.1) | 69 (42.9) | | 10.3 (9.2-11.0) | 1 (Ref) | | 1 (Ref) | | 25.8 (22.3-28.6) | 1 (Ref) | | 1 (Ref) | |
| G/G | 47 | 28 (63.6) | 16 (36.4) | | 9.5 (8.6-11.3) | 0.80 (0.55-1.17) | | 0.77 (0.52-1.15) | | 37.6 (19.8-48.6) | 0.63 (0.42-0.95) | | 0.62 (0.41-0.94) | |
| Control cohort (FOLFIRI, Cet) | | | | .68 | | | .78 | | .72 | | | .52 | | .34 |
| Any A | 103 | 73 (76.0) | 23 (24.0) | | 12.8 (10.0-14.1) | 1 (Ref) | | 1 (Ref) | | 52.0 (42.1-67.4) | 1 (Ref) | | 1 (Ref) | |
| G/G | 26 | 20 (80.0) | 5 (20.0) | | 12.2 (7.9-15.8) | 0.93 (0.58-1.51) | | 1.10 (0.65-1.85) | | 37.5 (24.5-56.2) | 1.27 (0.60-2.68) | | 1.50 (0.65-3.46) | |
| Combined *CD39 rs11188513* and *CD39 rs2226163* | | | | | | | | | | | | | | |
| Discovery cohort (FOLFIRI, Bev) | | | | .65 | | | .049 | | .14 | | | .040 | | .095 |
| Group 1 | 24 | 14 (58.3) | 10 (41.7) | | 11.6 (8.6-20.5) | Ref | | Ref | | 49.9 (21.5-NE) | Ref | | Ref | |
| Group 2 | 18 | 13 (72.2) | 5 (27.8) | | 13.5 (9.7-28.9) | 0.68 (0.32-1.46) | | 0.66 (0.30-1.43) | | 51.1 (23.7-NE) | 0.75 (0.25-2.29) | | 0.84 (0.27-2.61) | |
| Group 3 | 63 | 38 (63.3) | 22 (36.7) | | 11.3 (9.7-12.4) | 1.43 (0.80-2.54) | | 1.32 (0.71-2.45) | | 27.4 (19.4-40.0) | 1.94 (0.90-4.19) | | 1.95 (0.87-4.36) | |

**Table 1 Continued**

| SNP | Patients (n) | Tumor Response[a] | | | PFS,[b] mo | | | | | OS,[b] mo | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Yes | No | *P* Value | Median (95% CI) | HR (95% CI) | *P* Value | Adjusted HR (95% CI) | *P* Value | Median (95% CI) | HR (95% CI) | *P* Value | Adjusted HR (95% CI) | *P* Value |
| Validation cohort (FOLFIRI, Bev) | | | | .69 | | | .28 | | .18 | | | .047 | | .048 |
| Group 1 | 47 | 28 (63.6) | 16 (36.4) | | 9.5 (8.6-11.3) | Ref | | Ref | | 37.6 (19.8-48.6) | Ref | | Ref | |
| Group 2 | 29 | 17 (60.7) | 11 (39.3) | | 11.6 (8.2-12.7) | 1.01 (0.59-1.73) | | 1.00 (0.57-1.75) | | 25.2 (16.4-33.0) | 1.36 (0.78-2.35) | | 1.33 (0.75-2.35) | |
| Group 3 | 134 | 74 (56.5) | 57 (43.5) | | 9.9 (8.8-11.0) | 1.30 (0.88-1.91) | | 1.39 (0.92-2.08) | | 25.8 (21.1-28.6) | 1.65 (1.09-2.48) | | 1.68 (1.10-2.55) | |
| Control cohort (FOLFIRI, Cet) | | | | .88 | | | .68 | | .62 | | | .52 | | .29 |
| Group 1 | 26 | 20 (80.0) | 5 (20.0) | | 12.2 (7.9-15.8) | Ref | | Ref | | 37.5 (24.5-56.2) | Ref | | Ref | |
| Group 2 | 21 | 14 (73.7) | 5 (26.3) | | 10.0 (8.0-15.2) | 1.25 (0.67-2.32) | | 1.10 (0.56-2.19) | | 28.7 (20.3-NE) | 1.05 (0.41-2.67) | | 1.10 (0.37-3.27) | |
| Group 3 | 80 | 58 (77.3) | 17 (22.7) | | 13.3 (10.3-15.1) | 1.02 (0.62-1.68) | | 0.86 (0.51-1.47) | | 52.0 (42.1-NE) | 0.72 (0.33-1.57) | | 0.62 (0.26-1.46) | |
| *CD73 rs2229523* | | | | | | | | | | | | | | |
| Discovery cohort (FOLFIRI, Bev) | | | | .30 | | | .81 | | .46 | | | .017 | | .026 |
| G/G | 46 | 26 (59.1) | 18 (40.9) | | 10.3 (9.2-13.5) | 1 (Ref) | | 1 (Ref) | | 23.7 (17.6-44.3) | 1 (Ref) | | 1 (Ref) | |
| Any A | 59 | 40 (69.0) | 18 (31.0) | | 11.7 (10.1-14.9) | 0.94 (0.59-1.50) | | 0.83 (0.51-1.36) | | 41.9 (28.1-55.5) | 0.50 (0.28-0.91) | | 0.49 (0.26-0.92) | |
| Validation cohort (FOLFIRI, Bev) | | | | .46 | | | .68 | | .66 | | | .68 | | .78 |
| G/G | 118 | 63 (55.3) | 51 (44.7) | | 9.6 (8.8-11.0) | 1 (Ref) | | 1 (Ref) | | 25.1 (19.8-29.1) | 1 (Ref) | | 1 (Ref) | |
| Any A | 89 | 52 (60.5) | 34 (39.5) | | 9.7 (8.8-11.7) | 0.94 (0.68-1.28) | | 0.93 (0.66-1.30) | | 30.2 (22.4-35.8) | 0.94 (0.68-1.28) | | 0.95 (0.68-1.34) | |
| Control cohort (FOLFIRI, Cet) | | | | .80 | | | .49 | | .38 | | | .88 | | .47 |
| G/G | 64 | 44 (75.9) | 14 (24.1) | | 12.8 (10.1-14.5) | 1 (Ref) | | 1 (Ref) | | 52.0 (36.4-NE) | 1 (Ref) | | 1 (Ref) | |
| Any A | 65 | 49 (77.8) | 14 (22.2) | | 12.3 (9.6-15.2) | 1.14 (0.78-1.68) | | 1.19 (0.81-1.77) | | 46.5 (37.5-67.4) | 1.05 (0.57-1.95) | | 1.27 (0.66-2.44) | |
| *A2BR rs2015353* | | | | | | | | | | | | | | |
| Discovery cohort (FOLFIRI, Bev) | | | | .22 | | | .055 | | .073 | | | .008 | | .004 |
| Any C | 84 | 50 (61.7) | 31 (38.3) | | 11.3 (9.8-12.2) | 1 (Ref) | | 1 (Ref) | | 28.1 (21.5-40.0) | 1 (Ref) | | 1 (Ref) | |
| T/T | 21 | 16 (76.2) | 5 (23.8) | | 15.3 (9.1-20.4) | 0.58 (0.32-1.04) | | 0.58 (0.32-1.05) | | 49.9 (28.8-68.7) | 0.34 (0.14-0.84) | | 0.24 (0.09-0.64) | |
| Validation cohort (FOLFIRI, Bev) | | | | .56 | | | .32 | | .66 | | | .54 | | .61 |
| Any C | 168 | 94 (58.0) | 68 (42.0) | | 9.5 (8.8-10.8) | 1 (Ref) | | 1 (Ref) | | 26.5 (21.1-32.0) | 1 (Ref) | | 1 (Ref) | |
| T/T | 39 | 24 (63.2) | 14 (36.8) | | 11.2 (9.6-13.4) | 0.82 (0.55-1.22) | | 0.91 (0.60-1.38) | | 26.4 (20.8-39.8) | 0.88 (0.58-1.33) | | 1.12 (0.72-1.74) | |
| Control cohort (FOLFIRI, Cet) | | | | .46 | | | .80 | | .94 | | | .71 | | .94 |

**Table 1** Continued

| SNP | Patients (n) | Tumor Response[a] | | | PFS,[b] mo | | | | OS,[b] mo | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Yes | No | *P* Value | Median (95% CI) | HR (95% CI) | *P* Value | Adjusted HR (95% CI) | Median (95% CI) | HR (95% CI) | *P* Value | Adjusted HR (95% CI) | *P* Value |
| Any C | 99 | 71 (76.3) | 22 (23.7) | | 11.8 (9.3-13.6) | 1 (Ref) | | 1 (Ref) | 42.8 (37.5-60.7) | 1 (Ref) | | 1 (Ref) | |
| T/T | 25 | 20 (83.3) | 4 (16.7) | | 14.0 (10.0-15.7) | 0.94 (0.59-1.51) | | 0.98 (0.58-1.64) | 49.8 (23.9-NE) | 0.86 (0.40-1.88) | | 0.97 (0.42-2.24) | |

Data presented as n or n (%).
Abbreviations: Bev = bevacizumab; Cet = cetuximab; CI = confidence interval; ECOG = Eastern Cooperative Oncology Group; FOLFIRI = 5-fluorouracil, leucovorin, oxaliplatin, irinotecan; HR = hazard ratio; NE = not estimable; OS = overall survival; PFS = progression-free survival; Ref = reference; SNP = single nucleotide polymorphism.
[a]*P* value computed using $\chi^2$ test.
[b]*P* value computed using log-rank test on univariate analysis and Wald test on multivariable Cox proportional hazards regression model adjusted for sex, ECOG performance status, liver-limited disease, and BRAF status in discovery and control cohorts and sex, age, ECOG performance status, primary tumor site, primary tumor resected, liver-limited disease, adjuvant chemotherapy, BRAF status, and RAS status in the validation cohort.

*rs2226163* was significantly associated with survival (Table 1). Patients with any C allele in *CD39 rs11188513* had significantly shorter median OS (25.8 vs. 31.6 months; HR, 1.50; 95% CI, 1.08-2.09; $P = .014$) on univariate analysis (Figure 2B), with consistent results on multivariable analysis (HR, 1.53; 95% CI, 1.09-2.15; $P = .013$). *CD39 rs11188513* did not show a statistically significant difference, although a trend was found for PFS on univariate (9.9 vs. 9.6 months; HR, 1.30; 95% CI, 0.94-1.80; $P = .100$) and multivariable (HR, 1.41; 95% CI, 1.00-1.98; $P = .052$) analysis. Furthermore, patients carrying the G/G variant in *CD39 rs2226163* had significantly longer median OS on univariate (37.6 vs. 25.8 months; HR, 0.63; 95% CI, 0.42-0.95; $P = .023$) and multivariable (HR, 0.62; 95% CI, 0.41-0.94; $P = .024$) analysis (Supplemental Figure 1; available in the online version). This SNP was also evaluated in the discovery cohort and showed the same trend for OS on univariate and multivariable analysis. Patients carrying the G/G variant in *CD39 rs2226163* had longer median OS on univariate (49.9 vs. 28.6 months; HR, 0.62; 95% CI, 0.29-1.33; $P = .21$) and multivariable (HR, 0.61; 95% CI, 0.28-1.35; $P = .22$) analysis.

### *Evaluation of Predictive and Prognostic Effect of Adenosine-related SNPs in the Control Cohort*

In the control cohort, we found no evidence for associations of the identified SNPs (*CD39 rs11188513*, *CD39 rs2226163*, *CD73 rs2229523*, and *A2BR rs2015353*) with PFS or OS on univariate and multivariable analysis. The opposite trends to the results in the discovery and validation cohorts were observed in the *CD39 rs11188513* and *CD39 rs2226163* SNPs, in which the patients with any C allele in *CD39 rs11188513* had longer median OS on univariate (52.0 vs. 37.5 months; HR, 0.70; 95% CI, 0.37-1.32; $P = .26$) and multivariable (HR, 0.59; 95% CI, 0.31-1.14; $P = .12$) analysis. Also, patients with the G/G allele in *CD39 rs2226163* had shorter median OS on univariate (37.5 vs. 52.0 months; HR, 1.27; 95% CI, 0.60-2.68; $P = .52$) and multivariable (HR, 1.50; 95% CI, 0.65-3.46; $P = .34$) analysis (Table 1).

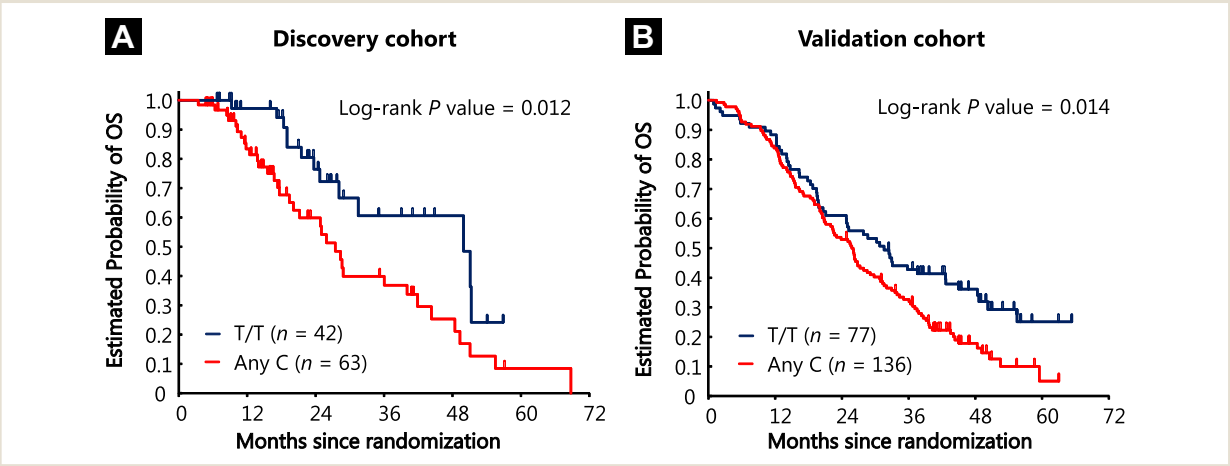
### *Combination of* CD39 rs11188513 *and* CD39 rs2226163

To further our understanding of the effect of 2 SNPs from the *CD39* gene, we combined *CD39 rs11188513* and *CD39 rs2226163* as follows: group 1, patients with T/T in *CD39 rs11188513* and G/G in *CD39 rs2226163*; group 2, patients with T/T in *CD39 rs11188513* and any A in *CD39 rs2226163*; and group 3, patients with any C in *CD39 rs11188513* and any A in *CD39 rs2226163*. In the discovery cohort, group 3 patients showed significantly shorter PFS and OS ($P = .049$ and $P = .040$, respectively) on the univariate analysis. However, the effect was no longer statistically significant on multivariable analysis. In the validation cohort, patients in group 3 still showed a significant association with shorter OS on both univariate and multivariable analysis ($P = .047$ and $P = .048$, respectively). This effect was not found in the control cohort (Table 1).

## Discussion

We tested the hypothesis that the adenosine-related SNPs are associated with the efficacy of bevacizumab-based chemotherapy in patients with mCRC. Our data showed that the SNPs in CD39,

**Figure 2** Overall Survival of Patients With *CD39 rs11188513* Variants, T/T or Any C (T/C or C/C) in the (A) Discovery Cohort and (B) Validation Cohort




CD73, and A2BR were significantly associated with the clinical outcomes in patients with mCRC treated with FOLFIRI plus bevacizumab as first-line treatment. *CD39 rs11188513* was a strong prognosticator and was validated in 3 independent cohorts.

Although adenosine-related molecules have been examined in various cancers, their relationship with the efficacy of anti-VEGF therapy has not been studied. Extracellular adenosine, produced via CD39 and CD73, not only stimulates cancer cells through A2BR but also regulates tumor-infiltrating immune cells through A2AR and A2BR.[4] Consistent with this, the loss of CD39, CD73, A2AR, or A2BR in mice models reportedly leads to activation of antitumor immunity and tumor resistance.[8,21-23] Furthermore, under hypoxic states, CD39 and CD73 upregulation through HIF-1α contributes to adenosine production[15,16] and might, therefore, serve as a resistance mechanism to antiangiogenic therapy. The Tregs and M2-type macrophage reportedly stimulate angiogenesis through adenosine-A2AR/A2BR signals in mice models,[24,25] implying that the adenosine pathway is an important alternative proangiogenic pathway. These findings are consistent with our data that the adenosine pathway is not only prognostic but also predictive for bevacizumab-based chemotherapy.

To the best of our knowledge, the role of CD39 polymorphisms in regulating angiogenesis and clinical outcome in cancer patients has not been previously reported. Both *CD39 rs11188513* and *CD39 rs2226163* are in the 3′-UTR region of the gene and are considered binding sites for microRNA (miR). Liu et al[26] reported that miR-155 expression was proportional to peripheral CD39-expressed Tregs in sepsis patients.[26] Zhao et al[27] showed a close inverse relationship between miR-142-3p and CD39 expression on Tregs in healthy mouse and human models. These indicate that SNPs in the binding site of miR-155 and miR-142-3p might be regulating the function of CD39. In addition, the 2 SNPs might work as tag SNPs and influence functional effects through related polymorphisms at other loci of CD39. In our present study, *CD39 rs11188513* was associated, not only with OS, but also with PFS. Although the influence of SNPs in CD39 on phenotypic change is still unknown, it is considered that the gain of function helps develop resistance to anti-VEGF–based chemotherapy.

Our study showed that the SNPs in CD39 had opposite trends in patients treated with cetuximab-based chemotherapy. These findings are in agreement with data from Zhi et al,[28] who showed that the expression of CD73 and epidermal growth factor receptor (EGFR) had positive correlations in breast cancer clinical samples and that EGFR expression was decreased by suppressing, not only CD73 or A2AR, but also adenosine. In colorectal cancer, Wu et al.[29] showed that adenosine increased the expression of EGFR. Furthermore, Cushman et al[17] demonstrated that high CD73 expression was associated with longer PFS in mCRC patients who underwent cetuximab-based chemotherapy, using clinical samples from the CALGB (Cancer and Leukemia Group B) 80203 study (n = 238),[17] consistent with our data. The gain of function status from the CD39 SNPs might result in better survival with cetuximab-based chemotherapy than with bevacizumab-based chemotherapy in patients with mCRC.

The clinical significance of CD73 and A2BR polymorphisms in cancer patients also remains unknown. *CD73 rs2229523* is a common missense SNP, which shows A1682G as a base pair change and Thr376Ala as an amino acid change. Similarly, *A2BR rs2015353* is a common missense SNP, which shows T437C as a base pair change and Ser9Pro as an amino acid change. Figler et al[30] demonstrated that the *A2BR* messenger RNA levels in macrophages and *A2BR rs2015353* showed significant correlations with interleukin-6 production in patients with diabetes, suggesting that the SNP can change the function of A2BR. Furthermore, this SNP might affect functional activity as a tag SNP. According to our experimental data, both *CD73 rs2229523* and *A2BR rs2015353* were strongly associated with prognosis in the discovery cohort. Further studies are necessary to confirm the clinical significance of these SNPs.

Based on the accumulating evidence, this adenosine pathway could be crucial for cancer progression and metastasis. Clinical trials of inhibitors of the adenosine pathway are ongoing. Although the

antibodies for CD39 or A2BR are still in the preclinical stage, an anti-CD73 monoclonal antibody, MEDI9447 (ClinicalTrials.gov identifier, NCT02503774),[31] and antagonists for the A2AR, CPI-444 (ClinicalTrials.gov identifier, NCT02655822) or PBF-509 (ClinicalTrials.gov identifier, NCT02403193),[32] are currently in phase I clinical trials for solid cancers. Activation of A2AR reportedly promotes the expression of CTLA-4 and PD-1 on T cells and enhances immunosuppressive function.[13,33] In contrast, anti−PD-1 therapy or adoptive T-cell therapy for cancer patients stimulates the expression of CD73, suggesting a possible resistance mechanism.[34] Recent reports have also shown that anti-adenosine pathway therapy can cooperate with other existing immune checkpoint inhibitors such as anti−CTLA-4 or anti−PD-1 in preclinical studies.[13,35,36] Following the preclinical results, the previously cited phase I clinical trials (ClinicalTrials.gov identifiers, NCT02503774, NCT02655822, and NCT0240319) are evaluating the clinical effects of the combination of anti−CD73/anti−A2AR drugs and PD-1/programmed cell death ligand 1 inhibitors. It would be warranted to examine whether the selected SNPs might be potential biomarkers, not only for the novel adenosine-related drugs, but also for the existing immunotherapies.

The present study had limitations. A selection bias could not be excluded owing to the retrospective study design. Although *CD39 rs2226163*, *CD73 rs2229523*, and *A2BR rs2015353* were prognostic markers in 1 cohort, the results were not validated in the other cohort. This result might have been due to the differences in the baseline characteristics (Supplemental Table 2; available in the online version). Nonetheless, our results convincingly support the previous findings from 2 prospective phase III clinical trials, FIRE-3 and TRIBE. Furthermore, the SNPs were more strongly related to OS than to PFS and not to the tumor response. Hence, it is unclear whether these SNPs are prognostic or predictive and whether these results can be attributed mainly to the effect of bevacizumab or to subsequent immune modulation. Further functional studies and large-scale prospective studies are warranted to fully elucidate our results.

## Conclusion

To the best of our knowledge, the present study is the first to show the associations of genetic variants in CD39, CD73, and A2BR with the clinical outcomes of patients with mCRC treated with FOLFIRI plus bevacizumab. We found that the patients with the C allele of *CD39 rs11188513* had much worse OS than did the others in the bevacizumab cohort and might have survival benefits, not from bevacizumab-based chemotherapy, but from cetuximab-based chemotherapy as first-line treatment. If our findings are validated prospectively, this SNP could be predictive for those patients with mCRC who would benefit from bevacizumab-based chemotherapy.

### *Clinical Practice Points*

- Adenosine has a potent immunosuppressive and angiogenic ability in the tumor microenvironment.
- Although some reports have shown that the expression of adenosine-related molecules has a strong association with the survival of cancer patients, the predictive or prognostic role of genetic changes within adenosine-related molecules remains unknown.
- Using data from 451 mCRC patients from phase III clinical trials (FIRE-3 and TRIBE), our study tested the hypothesis that the SNPs in adenosine-related molecules are associated with immune dysregulation and the efficacy of bevacizumab.
- We investigated relationships between the SNPs and clinical outcomes in patients with mCRC who underwent bevacizumab-based chemotherapy, with the cetuximab-based chemotherapy group as the control.
- The patients with any C allele in *CD39 rs11188513* had significantly shorter median OS, which was confirmed in the discovery and validation cohorts.
- The patients with any A allele in *CD73 rs2229523* had significantly longer median OS; the patients carrying the T/T variant in *A2BR rs2015353* had significantly longer median OS; and the patients carrying the G/G variant in *CD39 rs2226163* had significantly longer median OS in 1 cohort.
- The SNPs in *CD39* had opposite results in the patients who underwent bevacizumab-based chemotherapy versus cetuximab-based chemotherapy.
- Our results suggest that the selected SNPs in adenosine-related molecules could be biomarkers for bevacizumab-based chemotherapy and promising therapeutic targets in mCRC.

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## Disclosure

The authors declare that they have no competing interests.

## Supplemental Data

Supplemental figure and tables accompanying this article can be found in the online version at https://doi.org/10.1016/j.clcc.2018.09.003.

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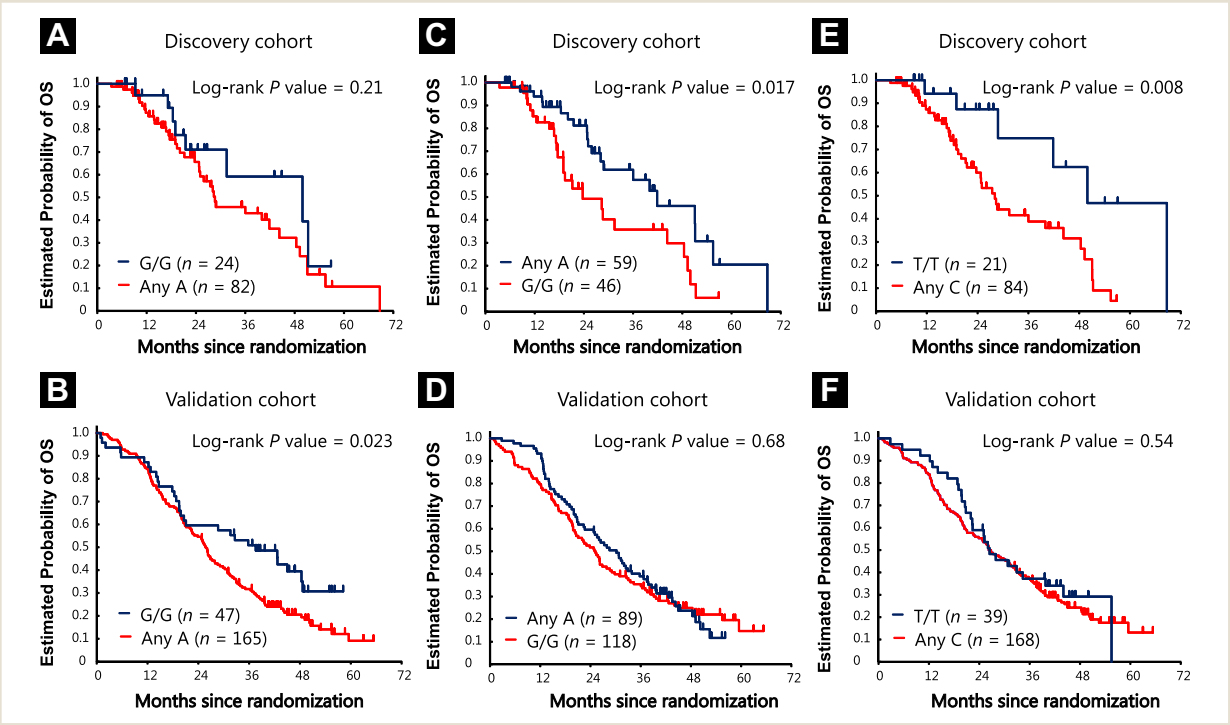
**Supplemental Figure 1** Overall Survival (OS) of Patients With *CD39 rs2226163* Variants, G/G or Any A (G/A or A/A), With *CD73 rs2229523* Variants, Any A (G/A or A/A) or G/G, and With *A2BR rs2015353* Variants, T/T or Any C (T/C or C/C), in (A, C, E) Discovery Cohort and (B, D, F) Validation Cohort, Respectively



**Supplemental Table 1** Selected SNPs in Adenosine-related Molecules and Their Characteristics

| Adenosin-related Molecule | Encoding Gene | Chromosome | SNP (rs Number) | Location of Polymorphism | Global MAF | Base Exchange | Predictive Function or Reported Data |
|---|---|---|---|---|---|---|---|
| CD39 | *ENTPD1* | 10 | rs11188513 | 3′ UTR | 0.4161 | C > T | MicroRNA binding, tag SNP |
| | | | rs2226163 | 3′ UTR | 0.4756 | A > G | MicroRNA binding, tag SNP |
| CD73 | *NT5E* | 6 | rs2229523 | Exon (missense) | 0.2067 | A > G | Changing protein expression |
| A2AR | *ADORA2A* | 22 | rs5751876 | Exon (synonymous) | 0.4423 | T > C | Methotrexate response in rheumatoid arthritis |
| A2BR | *ADORA2B* | 17 | rs2015353 | Exon (missense) | 0.3712 | T > C | Tag SNP |
| HIF-1α | *HIF1A* | 14 | rs2057482 | 3′ UTR | 0.2424 | T > C | MicroRNA binding, tag SNP |
| | | | rs11549465 | Exon (missense) | 0.0731 | C > T | Increasing risk of prostate cancer, tag SNP |

Abbreviations: HIF-1α = hypoxia-inducible factor-1α; MAF = minor allele frequency; SNP = single nucleotide polymorphism; UTR = untranslated region.

**Supplemental Table 2** Comparison of Baseline Characteristics Between FIRE-3 FOLFIRI Plus Bevacizumab, TRIBE FOLFIRI Plus Bevacizumab, and FIRE-3 FOLFIRI Plus Cetuximab Arms (n = 451)

| Characteristic | Discovery Cohort (FIRE-3: FOLFIRI, Bevacizumab; n = 107) | Validation Cohort (TRIBE: FOLFIRI, Bevacizumab, *n* = 215) | Control Cohort (FIRE-3: FOLFIRI, Cetuximab; n = 129) | *P* Value [a] |
|---|---|---|---|---|
| Sex | | | | .013 |
| Male | 70 (65) | 132 (61) | 99 (77) | |
| Female | 37 (35) | 83 (39) | 30 (23) | |
| Age, y | | | | .006 |
| ≤ 65 | 62 (58) | 156 (73) | 75 (58) | |
| > 65 | 45 (42) | 59 (27) | 54 (42) | |
| ECOG PS | | | | <.001 |
| 0 | 56 (52) | 177 (82) | 80 (62) | |
| 1 | 51 (48) | 37 (17) | 49 (38) | |
| Unknown[b] | 0 (0) | 1 (1) | 0 (0) | |
| Tumor location | | | | .157 |
| Right side | 25 (23) | 53 (25) | 22 (17) | |
| Left side | 81 (76) | 147 (68) | 105 (81) | |
| Unknown[b] | 1 (1) | 15 (7) | 2 (2) | |
| Liver-limited disease | | | | .615 |
| No | 75 (70) | 150 (70) | 84 (65) | |
| Yes | 32 (30) | 65 (30) | 45 (35) | |
| Primary tumor resected | | | | <.001 |
| No | 12 (11) | 80 (37) | 23 (18) | |
| Yes | 95 (89) | 135 (63) | 106 (82) | |
| Adjuvant chemotherapy | | | | .131 |
| No | 86 (80) | 188 (87) | 103 (80) | |
| Yes | 21 (20) | 27 (13) | 25 (19) | |
| Unknown[b] | 0 (0) | 0 (0) | 1 (1) | |
| KRAS status | | | | <.001 |
| Wild type | 103 (96) | 88 (41) | 125 (97) | |
| Mutant | 4 (4) | 90 (42) | 4 (3) | |
| Unknown[b] | 0 (0) | 37 (17) | 0 (0) | |
| RAS status | | | | <.001 |
| Wild type | 66 (62) | 50 (23) | 83 (64) | |
| Mutant | 17 (16) | 110 (51) | 19 (15) | |
| Unknown[b] | 24 (22) | 55 (26) | 27 (21) | |
| BRAF status | | | | .271 |
| Wild type | 81 (76) | 168 (78) | 95 (74) | |
| Mutant | 4 (4) | 10 (5) | 7 (5) | |
| Unknown[b] | 22 (21) | 37 (17) | 27 (21) | |

Data presented as n (%).
Abbreviations: ECOG = Eastern Cooperative Oncology Group; FOLFIRI = 5-fluorouracil, leucovorin, oxaliplatin, irinotecan; PS = performance status.
[a] *P* value computed using $\chi^2$ test for categorical factors.
[b] Unknown group was not included in the present analysis.