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# Clinical Impact of Primary Tumor Location and *RAS*, *BRAF* V600E, and *PIK3CA* Mutations on Epidermal Growth Factor Receptor Inhibitor Efficacy as Third-line Chemotherapy for Metastatic Colorectal Cancer

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**Abstract.** *Background/Aim: Primary tumor location and RAS and BRAF V600E mutations are predictors of the efficacy of epidermal growth factor receptor (EGFR) inhibitors. However, there are limited reports on their effects on the outcomes of third-line chemotherapy with EGFR inhibitors in metastatic colorectal cancer (mCRC) patients. Patients and Methods: We retrospectively collected the clinical data of KRAS exon 2 wild type (WT) mCRC patients treated with EGFR inhibitor monotherapy or EGFR inhibitor plus irinotecan as third-line chemotherapy. The association between primary tumor location, RAS (KRAS exon 3, 4 or NRAS), BRAF V600E, and PIK3CA mutational status, and treatment outcome was evaluated. Results: A total of 72 patients were included in this study. In multivariate analysis, RAS (p=0.004) and BRAF mutations (p=0.00008) were independent factors for shorter PFS. Poor performance status (p=0.01) and BRAF mutation (p=0.00002) were independent factors for shorter OS, whereas primary tumor location and PIK3CA mutation did not influence survival. Conclusion: Additional analysis of RAS and BRAF mutations could contribute to the selection of patients who are likely to benefit from third-line EGFR inhibitors, regardless of primary tumor location.*

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*Key Words:* Epidermal growth factor receptor inhibitor, metastatic colorectal cancer, *RAS* mutation, *BRAF* V600E mutation, primary tumor location, third-line treatment.

In the current treatment strategy for metastatic colorectal cancer (mCRC), the presence of *RAS* or *BRAF* gene mutations and primary tumor location are widely used biomarkers for selecting a targeted therapeutic treatment (1, 2). The prognosis of right-sided mCRC is well known to be worse than that of left-sided mCRC (3). One of the reasons is that right-sided mCRC has a higher incidence of the *BRAF* V600E mutation, which is a negative predictor for the efficacy of epidermal growth factor receptor (EGFR) inhibitors (4-7) and is characterized by rapid disease progression (7-9). In contrast, the *PIK3CA* mutation, which is more frequent in the right-sided mCRC, is controversial in terms of whether it is a predictor for the chemotherapeutic effect of EGFR inhibitors (5, 10-13).

Initially, primary tumor location was drawing attention as a predictor of the efficacy of EGFR inhibitors in the post-hoc analysis of the NCIC CO.17 trial (14). Subsequently, Arnold *et al.* reported a post-hoc analysis of large-scale randomized clinical trials [CRYSTAL (15), FIRE-3 (16), CALGB/SWOG 80405 (17), PRIME (18), PEAK (19), and 20050181 (20)] to examine the relationship between the primary tumor location, prognosis, and the chemotherapeutic effect of molecularly targeted agents in front-line chemotherapy (21). The hazard ratio (HR) of progression-free survival (PFS) and overall survival (OS) in mCRC patients with right-sided tumors was worse than that of those with left-sided tumors, indicating that right-sided tumors had a worse prognosis (21). In addition, in terms of the efficacy of chemotherapy (PFS and OS), the HRs of chemotherapy with EGFR inhibitors were significantly better than those of chemotherapy with vascular endothelial growth factor (VEGF) inhibitors in mCRC patients with left-sided tumors (21). Based on the results of the analysis, chemotherapy with an EGFR inhibitor is recommended for *RAS*/*BRAF* wild type

(WT) mCRC patients with left-sided tumors, and chemotherapy with a VEGF inhibitor is recommended for *RAS*/*BRAF* WT mCRC patients with right-sided tumors or *RAS*/ *BRAF* mutant (MT) mCRC patients (1, 2).

In clinical practice, *RAS*/*BRAF* WT mCRC patients with right-sided tumors are treated with chemotherapy plus VEGF inhibitor as first-line chemotherapy, and EGFR inhibitors are considered in the third line of treatment because they do not add to OS as the second-line treatment (20, 22). However, there are only a few detailed reports about the relationship between primary tumor location, *RAS*, *BRAF* V600E, and *PIK3CA* mutations, and the chemotherapeutic effects of EGFR inhibitors in third-line treatment compared to those in the first-line treatment (23). This study aimed to investigate the effects of primary tumor location and the mutations in the abovementioned genes on the treatment outcome with EGFR inhibitors as third-line chemotherapy.

## Patients and Methods

*Patients*. A total of 219 mCRC patients treated with EGFR inhibitor monotherapy or EGFR inhibitor plus irinotecan as third-line chemotherapy, at the Cancer Institute Hospital of the Japanese Foundation for Cancer Research, from April 2011 to September 2017 were retrospectively enrolled in this study. Clinical characteristics including patient background factors, laboratory and imaging data, and prognosis were collected, and the follow-up period ended (the time of data cut off) on March 12, 2020. This study was approved by the Institutional Review Board of the Japanese Foundation for Cancer Research (Tokyo, Japan, registry number 1098; approval number 2020-1310). The protocol was described on the hospital website and the subjects were provided with the opportunity to opt out; therefore, no new consent was required from patients. All methods were performed in accordance with the Declaration of Helsinki.

*Tumor tissue DNA sequencing*. Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissues obtained from biopsies or surgical specimens. We analyzed the *KRAS*, *NRAS*, *BRAF,* and *PIK3CA* genotypes using the bead-based multiplexed immunoassay system (xMAP Technology; Luminex, Tokyo, Japan). *KRAS* codon 12 and 13 mutations (MTs) (*KRAS* G12A, G12C, G12D, G12R, G12S, G12V, G13D) were detected using the MEBGEN *KRAS* Mutation Detection Kit (MBL, Tokyo, Japan), according to the manufacturer's instruction. MTs in *KRAS* codon 61 (Q61K, Q61E, Q61L, Q61P, Q61R, Q61H), codon 146 (A146T, A146S, A146P, A146E, A146V, A146G), and codon 600 (V600E), in *NRAS* codon 12 (G12S, G12C, G12R, G12D, G12V, G12A), codon 13 (G13S, G13C, G13R, G13D, G13V, G13A), and codon 61 (Q61K, Q61E, Q61L, Q61P, Q61R, Q61H), and in *PIK3CA* codon 542 (E542K), codon 545 (E545K), codon 546 (E546K), and codon 1047 (H1047R, H1047L) were detected using the GENOSEARCHTM Mu-PACKTM (MBL), according to the manufacturer's instructions.

*Treatment*. Irinotecan (CPT-11) plus cetuximab (C-mab) were administered intravenously at 150 mg/m$^2$ and 500 mg/m$^2$, respectively, every 2 weeks or as an initial dose of 400 mg/m$^2$ C-mab followed by weekly intravenous infusions of 250 mg/m$^2$ C-mab. CPT-11 plus panitumumab (P-mab) were administered intravenously every 2 weeks at 180 mg/m$^2$ and 6 mg/kg, respectively. C-mab monotherapy was administered at an initial dose of 400 mg/m$^2$ followed by weekly intravenous infusions of 250 mg/m$^2$ or 500 mg/m$^2$ C-mab every 2 weeks. P-mab monotherapy was administered intravenously every 2 weeks at a dose of 6 mg/kg P-mab. Dose reduction or interruption was performed based on the institutional standards of clinical practice.

*Study endpoints*. This study aimed to evaluate the relationship between clinical outcomes, primary tumor location and *RAS* (*KRAS* exon 3 or 4 and *NRAS)*, *BRAF* V600E, and *PIK3CA* mutations in mCRC patients treated with chemotherapy including EGFR inhibitor as the third-line treatment. The primary tumor location was divided into right-sided and left-sided tumors; right-sided tumors were defined as tumors arising anywhere from the cecum to the transverse colon and left-sided tumors were defined as tumors arising from the splenic flexure to the anorectal junction. PFS was defined as the time from the start of third-line treatment to the date of disease progression and OS was the time from third-line treatment initiation to the date of death. The response rate (RR) was calculated as the proportion of patients with complete response (CR) plus partial response (PR). Disease control rate (DCR) was calculated as the proportion of patients with CR and PR plus stable disease (SD). Objective responses were evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Imaging evaluation, mainly by computed tomography, was performed every 1.5-2 months following standard institutional practice.

*Statistical analysis*. PFS and OS were estimated using the Kaplan–Meier method. All reported *p*-values are the results of two-sided tests, with $p<0.05$ considered statistically significant. In the Cox proportional hazard analysis, factors with $p<0.05$ in the univariate analysis were included in the multivariate analysis (backward stepwise methods). The statistical software EZR (Easy R, Y Kanda, Jichi Medical University Saitama Medical Center, Saitama, Japan), which is based on R and R commander, was used for all the statistical analyses (24).

## Results

*Patient characteristics*. A total of 219 patients who received EGFR inhibitor monotherapy or EGFR inhibitor plus irinotecan as third-line chemotherapy and C-mab were enrolled. Among them, 72 patients were eligible for this study (Figure 1). Patient clinical characteristics are shown in Table I. The median age at the initiation of the third line chemotherapy was 64.0 years (range=33.0-85.0 years). Of the 72 patients, 36 were male (50.0%). The sites of metastases were liver (50; 69.4%), followed by the lungs (32; 44.4%), intraabdominal lymph nodes (33; 45.8%), and peritoneum (20; 27.8%). Precisely, 59 (81.9%) patients received EGFR inhibitor plus irinotecan and 13 (18.1%) received EGFR inhibitor monotherapy. Thirty-one patients (43.0%) had right-sided tumors. *KRAS* exon 3, *KRAS* exon 4, *NRAS*, *BRAF,* and *PIK3CA* mutations were present in 0%, 6.9%, 2.8%, 12.5%, and 12.8% of the tumors, respectively

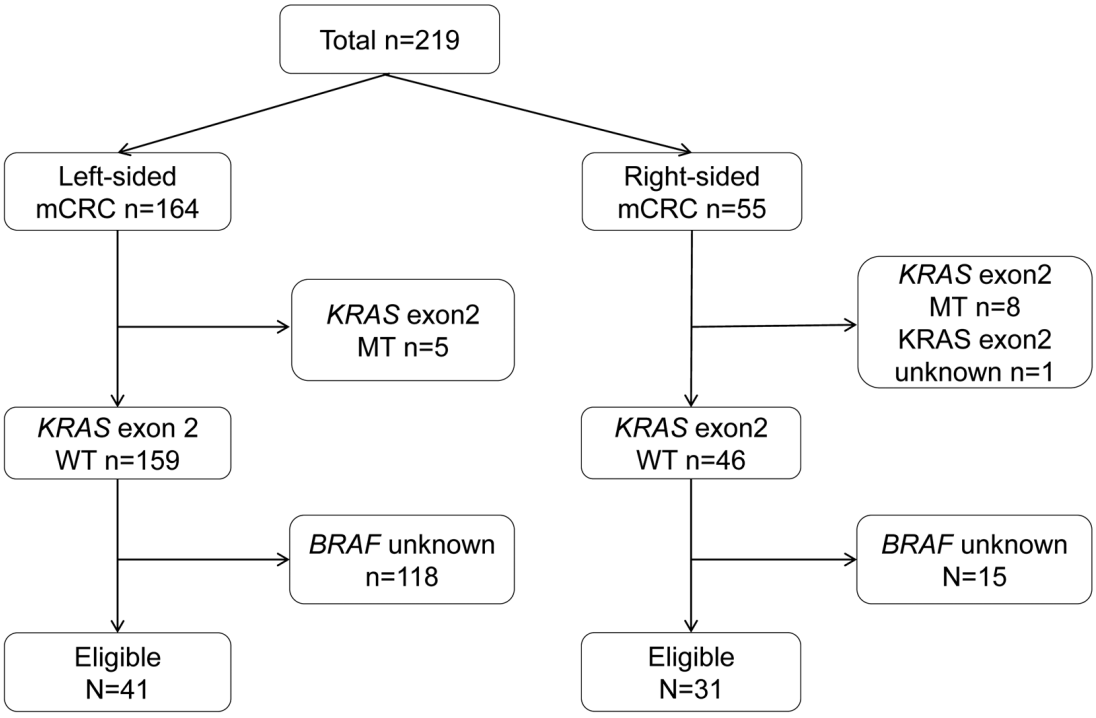



Figure 1. *Study design. Databases were queried for patients who were treated with EGFR inhibitor with or without irinotecan as third-line chemotherapy from April 2011 to September 2017. Patients with KRAS exon 2 mutation or BRAF V600E status unknown were excluded. The remaining patients were eligible for this study. EGFR: Epidermal growth factor receptor; mCRC: metastatic colorectal cancer; MT: mutations; WT: wild type.*

(Table I). The frequency of *BRAF* V600E and *PIK3CA* mutations was higher in patients with right-sided tumors than in those with left-sided tumors (*BRAF* V600E: 22.6% *vs*. 4.9%, $p$=0.03; *PIK3CA*: 19.2% *vs*. 4.8%, $p$=0.2, Table II).

*Response and survival endpoints*. RR, DCR, median PFS, and OS in all patients were 27.8%, 70.8%, 6.1 months (4.2-6.9), and 12.7 months (11.4-15.2), respectively. The ORR and DCR according to the *RAS* and *BRAF* V600E mutations and primary tumor location are shown in Figure 2. When mCRC patients with gene mutations were excluded, both ORR and DCR were elevated regardless of the primary tumor location (ORR: *RAS* WT 29.2%, *RAS*/*BRAF* WT 33.3%; DCR: *RAS* WT 75.4%, *RAS*/*BRAF* WT 84.2%, Figure 2A-C). The PFS of the *RAS* WT subgroup was also significantly longer than that of the *RAS* MT subgroup (6.5 *vs*. 2.3 months, $p$=0.009) irrespective of the primary tumor location (Figure 3A). In addition, the PFS and OS of the *BRAF* V600E WT subgroup were significantly longer than those of the *BRAF* V600E MT subgroup (PFS: 6.5 *vs*. 1.6 months, $p$=0.00007; OS: 13.4 *vs*. 2.9 months, $p$<0.00001) irrespective of the primary tumor location (Figure 3B). Furthermore, PFS of the *RAS*/*BRAF* WT subgroup was significantly longer than that of any MT subgroup (PFS: 6.8 *vs*. 1.9 months, $p$=0.000001) irrespective of the primary tumor location (Figure 3C). Similar to the response results, both the PFS and OS in the *RAS* WT or *RAS*/*BRAF* WT subgroup were longer than those in the *KRAS* exon 2 WT subgroup (median PFS: *RAS* WT 6.5 months, *RAS*/*BRAF* WT 6.8 months; OS: *RAS* WT 12.7 months, *RAS*/*BRAF* WT 14.0 months).

*Univariate and multivariate analyses of predictors of clinical outcomes*. In the univariate Cox proportional hazard analysis, lung metastasis, carbohydrate antigen 19-9 (CA 19-9) level, *RAS* mutation, and *BRAF* V600E mutation were predictors for PFS, and performance status (PS), lung metastasis, and *BRAF* V600E mutation were predictors for OS (Table III).

In the multivariate analysis, *RAS* and *BRAF* V600E mutations were independent factors for shorter PFS (*RAS* mutation: HR=3.46, $p$=0.004; *BRAF* V600E mutation: HR=4.44, $p$=0.00008, Table III). PS and *BRAF* V600E mutation were independent factors for shorter OS (PS: HR=2.18, $p$=0.01; *BRAF* V600E mutation: HR=6.06, $p$=0.00002, Table III). On the other hand, primary tumor location (PFS: HR=1.22, $p$=0.40; OS: HR=1.22, $p$=0.42 in univariate analysis), and *PIK3CA* mutation (PFS: HR=1.14, $p$=0.77; OS: HR=0.82, $p$=0.67 in univariate analysis) did not influence survival (Table III).

Table I. *Patient demographics and clinical characteristics*.

| Characteristics | Total (N=72) No. of patients (%) |
|---|---|
| Age at enrollment, years | |
| Median [range] | 64.0 [33.0-85.0] |
| Gender | |
| Male | 36 (50.0) |
| Female | 36 (50.0) |
| ECOG PS | |
| /0 | 57 (79.2) |
| /1 | 14 (19.4) |
| /2 | 1 (1.4) |
| Treatment at the time of enlorment | |
| Irinotecan+Cetuximab | 57 (79.1) |
| Irinotecan+Panitumumab | 2 (2.8) |
| Cetuximab monotherapy | 3 (4.2) |
| Panitumumab monotherapy | 10 (13.9) |
| Primary site | |
| Cecum | 2 (2.8) |
| Ascending colon | 15 (20.8) |
| Transverse colon | 14 (19.4) |
| Discending colon | 4 (5.6) |
| Sigmoid colon | 22 (30.6) |
| Rectum | 15 (20.8) |
| Metastatic site | |
| Liver | 50 (69.4) |
| Lung | 32 (44.4) |
| Lymph node | 33 (45.8) |
| Peritoneal | 20 (27.8) |
| Bone | 6 (8.3) |
| Other | 12 (16.7) |
| Tumor markers (at initiation of third-line chemotherapy) | |
| CEA median, [range] | 61.1 [1.1-19,638.9] |
| CA19-9 median, [range] | 54.0 [2-50,000] |
| Gene mutation | |
| *KRAS* exon 3 | 0 (0) |
| *KRAS* exon 4 | 5 (6.9) |
| *NRAS* | 2 (2.8) |
| *BRAF* V600E | 9 (12.5) |
| *PIK3CA* | 6 (12.8)* |
| *RAS* wild | 65 (90.3) |
| *RAS*/*BRAF* wild | 56 (77.8) |

*We could only include 47 patients in the evaluation of *PIK3CA* gene mutation. ECOG PS: Eastern Cooperative Oncology Group Performance Status; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9; *KRAS*: kirsten rat sarcoma viral oncogene homolog; *NRAS*: neuroblastoma rat sarcoma viral oncogene homolog; *BRAF*: raf murine sarcoma viral oncogene homolog B; *PIK3CA*: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *RAS*: rat sarcoma viral oncogene homolog.

Table II. *The incidence and distribution of KRAS exon 3, KRAS exon 4, NRAS, BRAF V600E, and PIK3CA mutations*.

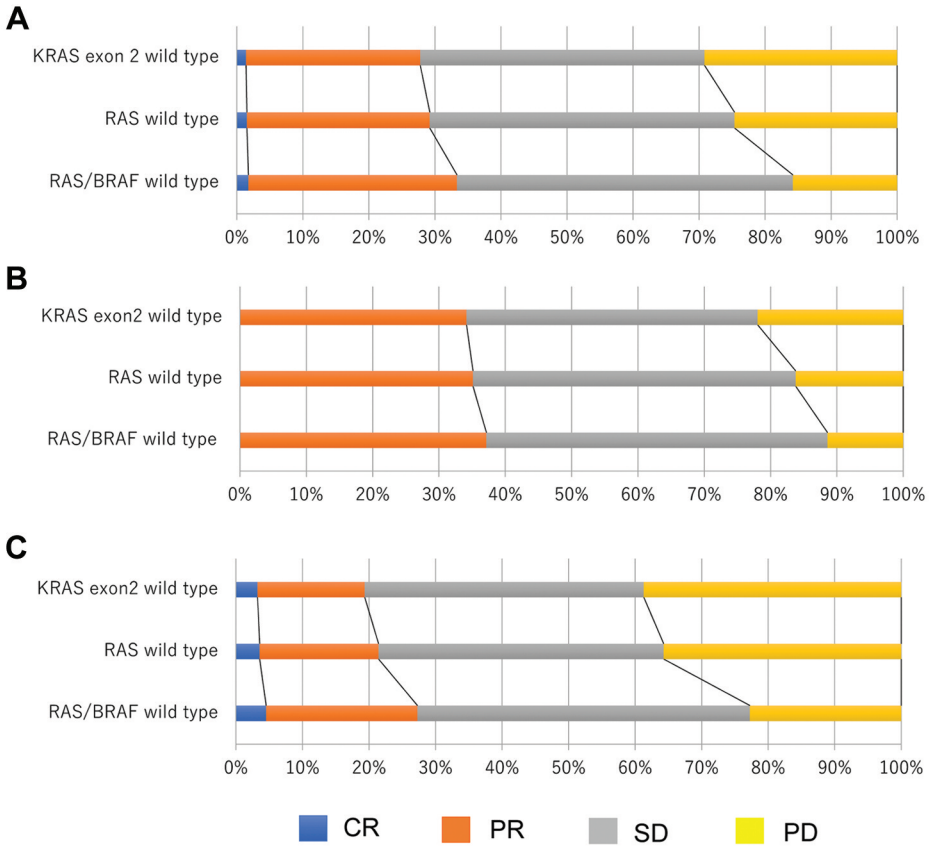
| Gene mutation | Left-sided tumors, n (%) | Right-sided tumors, n (%) | *p*-Value |
|---|---|---|---|
| *KRAS* exon 3 | 0 (0) | 0 (0) | 1 |
| *KRAS* exon 4 | 3 (7.3) | 2 (6.5) | 1 |
| *NRAS* | 1 (2.4) | 1 (3.2) | 1 |
| *BRAF* V600E | 2 (4.9) | 7 (22.6) | 0.03 |
| *PIK3CA* | 1 (4.8)* | 5 (19.2)* | 0.2 |

*We could only include 47 patients in the evaluation of *PIK3CA* gene mutation. *KRAS*: Kirsten rat sarcoma viral oncogene homolog; *NRAS*: neuroblastoma rat sarcoma viral oncogene homolog; *BRAF*: raf murine sarcoma viral oncogene homolog B; *PIK3CA*: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

## Discussion

The present study demonstrated that *RAS* and *BRAF* V600E mutations are predictors of shorter PFS, and *BRAF* V600E mutation is a predictor of shorter OS compared to the WT, although *PIK3CA* mutations and primary tumor location did not predict the clinical outcomes in *KRAS* exon 2 WT mCRC patients treated with EGFR inhibitors with or without CPT-11 as third-line chemotherapy.

Previously, in almost all reports on mCRC, the primary tumor location was divided into the rectum and colon. Therefore, since there are only a few reports discriminating between left-side and right-side primary tumor location especially in third-line treatment, it is difficult to evaluate the relationship between primary tumor location (left- and right-sided) and clinical outcomes of EGFR inhibitors. The summary of clinical outcomes of mCRC patients with right-sided tumors receiving later-line EGFR inhibitors is shown in Table IV (14, 23, 25, 26). Brule *et al*. examined whether the primary tumor location of colon cancer is a prognostic factor and a predictor of the benefits of C-mab using the results of the NCIC CO.17 trial (14). According to the results of this study, among *KRAS* exon 2 WT patients, those with left-sided tumors had a significantly improved PFS when treated with C-mab compared to best supportive care (BSC) (HR=0.28, $p<0.0001$), whereas those with right-sided tumors did not (HR=0.73, $p=0.26$, interaction $p=0.002$) (14). Furthermore, a recent additional analysis reported that patients with left-sided *RAS*/*BRAF* V600E WT tumors obtained a strong benefit of prolonged PFS following C-mab therapy (HR=0.20, $p<0.0001$), while right-sided tumors did not reach significance (HR=0.48; $p=0.16$) (25). Therefore, primary tumor location is a predictor of PFS benefit for C-mab therapy. On the other hand, Boeckx *et al*. reported the effect of primary tumor location on clinical outcomes of P-mab treatment in patients with *RAS* WT mCRC (23). A significant PFS benefit (HR=0.31; $p<0.0001$) was observed when P-mab was added to BSC for *RAS* WT left-sided mCRC patients (23). In contrast, no difference was observed in PFS of patients with right-sided tumors (HR=0.50;

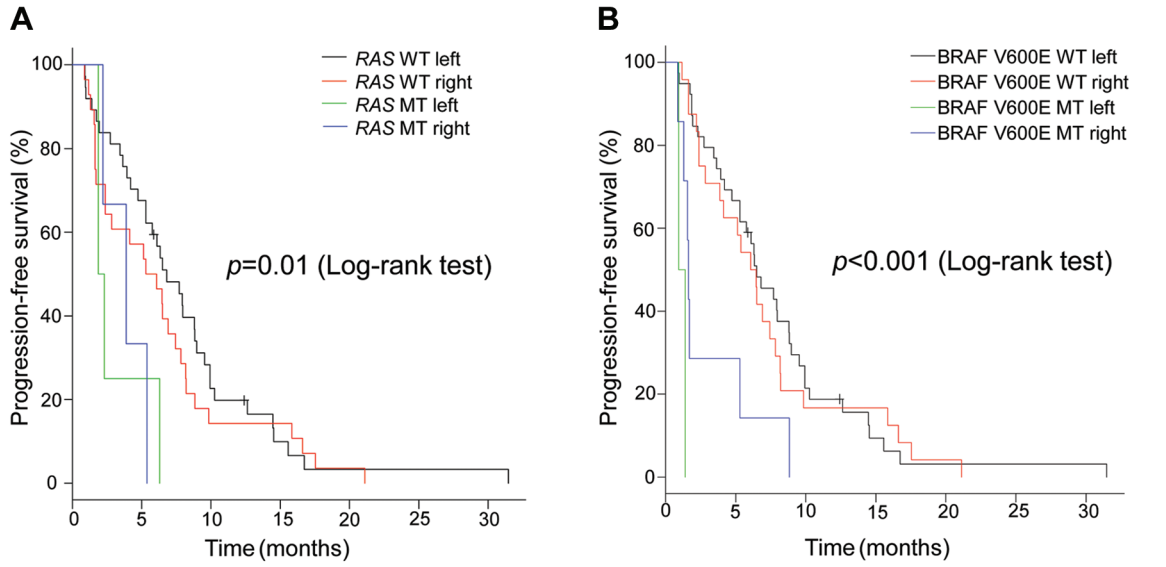



| | CR | PR | SD | PD | RR (%) | DCR (%) |
|---|---|---|---|---|---|---|
| *KRAS exon 2* WT, (n) | 1 | 19 | 31 | 21 | 27.8 | 70.8 |
| *RAS* WT, (n) | 1 | 18 | 30 | 16 | 29.2 | 75.4 |
| *RAS/BRAF* WT, (n) | 1 | 18 | 29 | 9 | 33.3 | 84.2 |

| | CR | PR | SD | PD | RR (%) | DCR (%) |
|---|---|---|---|---|---|---|
| *KRAS exon2* WT (n) | 0 | 14 | 18 | 9 | 34.1 | 78.0 |
| *RAS* WT (n) | 0 | 13 | 18 | 6 | 35.1 | 83.8 |
| RAS/BRAF WT (n) | 0 | 13 | 18 | 4 | 37.1 | 88.6 |

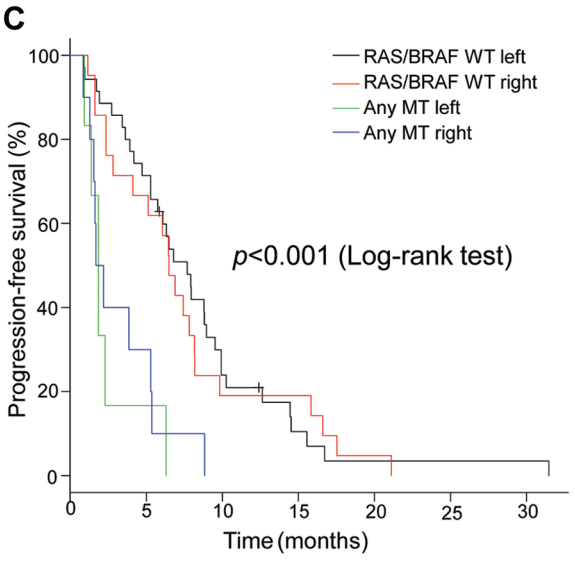
| | CR | PR | SD | PD | RR (%) | DCR (%) |
|---|---|---|---|---|---|---|
| *KRAS exon2* WT (n) | 1 | 5 | 13 | 12 | 19.4 | 61.3 |
| *RAS* WT (n) | 1 | 5 | 12 | 10 | 21.4 | 64.3 |
| RAS/BRAF WT (n) | 1 | 5 | 11 | 5 | 27.3 | 77.3 |

Figure 2. *Tumor response according to RAS/BRAF status. A) Tumor response in all metastatic colorectal cancer (mCRC) patients according to the RAS (KRAS exon 3,4 or NRAS) and BRAF V600E status. B) Tumor response in mCRC patients with left-sided tumors according to RAS and BRAF V600E status. C) Tumor response in mCRC patients with right-sided tumors according to RAS and BRAF V600E status. CR: Complete response; PR: partial response; SD: stable disease; PR: progressive disease; WT: wild type; RR: response rate; DCR: disease control rate.*




| | n | median | 95% CI |
|---|---|---|---|
| *RAS* WT left | 37 | 6.8 months | 5.3 to 8.8 |
| *RAS* WT right | 28 | 5.7 months | 2.4 to 7.4 |
| *RAS* MT left | 4 | 2.1 months | 1.9 to NA |
| *RAS* MT right | 3 | 3.9 months | 2.2 to NA |

| | n | median | 95% CI |
|---|---|---|---|
| *BRAF* V600E WT left | 39 | 6.5 months | 4.7 to 8.8 |
| *BRAF* V600E WT right | 24 | 6.3 months | 2.8 to 7.8 |
| *BRAF* V600E MT left | 2 | 1.2 months | 0.9 to NA |
| *BRAF* V600E MT right | 7 | 1.6 months | 0.9 to 5.3 |




| | n | median | 95% CI |
|---|---|---|---|
| *RAS/BRAF* WT left | 35 | 7.7 months | 5.3 to 9.0 |
| *RAS/BRAF* WT right | 21 | 6.5 months | 2.8 to 8.2 |
| Any MT left | 6 | 1.9 months | 0.9 to NA |
| Any MT right | 10 | 2.0 months | 0.9 to 5.3 |

Figure 3. *Kaplan–Meier estimates of progression-free survival (PFS) with respect to a) RAS (KRAS exon 3,4 or NRAS), b) BRAF V600E, c) RAS/BRAF V600E status and primary tumor location in mCRC patients treated with EGFR inhibitor with or without irinotecan as third-line chemotherapy. MT: Mutations; WT: wild type; CI: confidence interval; mCRC: metastatic colorectal cancer; EGFR: epidermal growth factor receptor. Comparison of PFS according to the RAS, BRAF V600E and RAS/BRAF V600E status (RAS or BRAF V600E or RAS/BRAF V600E wild vs. mutant) and primary tumor location (left-sided vs. right-sided). p-Values were calculated using log-rank test.*

Table III. *Cox proportional hazard analysis for factors associated with survival.*

| PFS | Univariate analysis | | | | Multivariate analysis | | | |
|---|---|---|---|---|---|---|---|---|
| | HR | Lower 95%CI | Upper 95%CI | *p*-Value | HR | Lower 95%CI | Upper 95%CI | *p*-Value |
| Gender (Female* or Male) | 1.16 | 0.72 | 1.89 | 0.54 | | | | |
| Age (<65* or ≥65 years) | 0.91 | 0.57 | 1.47 | 0.71 | | | | |
| Performance status (0* vs ≥1) | 1.54 | 0.87 | 2.76 | 0.14 | | | | |
| Primary tumor location (Left* or Right) | 1.22 | 0.76 | 1.98 | 0.40 | | | | |
| Liver metastasis (Negative* or Positive) | 0.98 | 0.58 | 1.65 | 0.94 | | | | |
| Lung metastasis (Negative* or Positive) | 0.61 | 0.37 | 0.99 | 0.045 | 0.67 | 0.40 | 1.12 | 0.13 |
| Peritoneal metastasis (Negative* or Positive) | 1.24 | 0.73 | 2.08 | 0.43 | | | | |
| Treatment regimen (EGFR inhibitor monotherapy* or with irinotecan) | 0.55 | 0.30 | 1.02 | 0.057 | | | | |
| CEA level before the chemotherapy (<5*, ≥5 ng/ml) | 1.51 | 0.64 | 3.52 | 0.34 | | | | |
| CA19-9 level before the chemotherapy (<37*, ≥37 U/ml) | 1.69 | 1.04 | 2.75 | 0.03 | 1.64 | 0.98 | 2.74 | 0.06 |
| *RAS* (*KRAS* exon 3 or 4 and *NRAS*) mutation (Negative* or Positive) | 2.90 | 1.26 | 6.67 | 0.01 | 3.46 | 1.48 | 8,08 | 0.004 |
| *BRAF* V600E mutation (Negative* or Positive) | 3.92 | 1.89 | 8.11 | 0.0002 | 4.44 | 2.12 | 9.28 | 0.00008 |
| *PIK3CA* mutation (Negative* or Positive) | 1.14 | 0.48 | 2.71 | 0.77 | | | | |
| OS | Univariate analysis | | | | Multivariate analysis | | | |
| | HR | Lower 95%CI | Upper 95%CI | *p*-Value | HR | Lower 95%CI | Upper 95%CI | *p*-Value |
| Gender (Female* or Male) | 0.81 | 0.51 | 1.32 | 0.41 | | | | |
| Age (<65* or ≥65 years) | 0.82 | 0.51 | 1.32 | 0.42 | | | | |
| Performance status (0* vs. ≥1) | 2.17 | 1.21 | 3,91 | 0.009 | 2.18 | 1.20 | 3,94 | 0.01 |
| Primary tumor location (Left* or Right) | 1.22 | 0.75 | 1.96 | 0.42 | | | | |
| Liver metastasis (Negative* or Positive) | 1.61 | 0.94 | 2.78 | 0.08 | | | | |
| Lung metastasis (Negative* or Positive) | 0.47 | 0.28 | 0.78 | 0.003 | 0.61 | 0.35 | 1.06 | 0.08 |
| Peritoneal metastasis (Negative* or Positive) | 1.17 | 0.69 | 1.97 | 0.56 | | | | |
| Treatment regimen (EGFR inhibitor monotherapy* or with irinotecan) | 1.04 | 0.54 | 2.00 | 0.91 | | | | |
| CEA level before the chemotherapy (<5*, ≥5 ng/ml) | 1.86 | 0.79 | 4,38 | 0.15 | | | | |
| CA19-9 level before the chemotherapy (<37*, ≥37 U/ml) | 1.42 | 0.87 | 2.31 | 0.16 | | | | |
| *RAS* (*KRAS* exon 3 or 4 and *NRAS*) mutation (Negative* or Positive) | 0.54 | 0.21 | 1.38 | 0.20 | | | | |
| *BRAF* V600E mutation (Negative* or Positive) | 5.95 | 2.66 | 13.3 | 0.00001 | 6.06 | 2.66 | 13.8 | 0.00002 |
| *PIK3CA* mutation (Negative* or Positive) | 0.82 | 0.32 | 2.09 | 0.67 | | | | |

*Reference. PFS: Progression-free survival; OS: overall survival; HR: hazard ratio; CI: confidence interval; EGFR: epidermal growth factor receptor; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9; *KRAS*: kirsten rat sarcoma viral oncogene homolog; *NRAS*: neuroblastoma rat sarcoma viral oncogene homolog; *BRAF*: raf murine sarcoma viral oncogene homolog B; *PIK3CA*: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

*p*=0.10) (22). Similar results were observed in *RAS*/*BRAF* WT patients receiving P-mab (23). However, the number of patients in both the studies was too small and insufficient for definitive conclusions. In the present study, in which CPT-11 plus EGFR inhibitor was used in more than 80% of the patients, the effect of EGFR inhibitor was observed in the right-sided tumors as well as the left-sided tumors, except for those with *RAS* and *BRAF* V600E mutations. Therefore, further studies are needed to clarify the effect of primary tumor location on the clinical outcomes of EGFR inhibitors as third-line chemotherapy.

In several guidelines for mCRC treatment, C-mab, P-mab, CPT-11 plus C-mab/P-mab, regorafenib, and trifluridine/tipiracil (FTD/TPI) (+bevacizumab) are recommended as third-line or later-line treatments. The summary of clinical outcomes of mCRC patients receiving later-line treatments is shown in Table V (27-36). Among them, regorafenib and TPI/FTD (+bevacizumab) have greatly expanded the options for the salvage treatment of mCRC (30, 33-35). However, the response rate of these therapies is very low (1.0%-4.0%), and they are aimed at disease progression and not at tumor shrinkage. Therefore, considering the perspective of response rate and survival, the use of EGFR inhibitors should be considered, even in mCRC patients with right-sided tumors except for those with *RAS* and *BRAF* V600E mutations. Currently, it is recommended to evaluate the *RAS* and *BRAF* status prior to first-line treatment (1, 2) but if only the results of *KRAS* exon 2 mutations are available, it is important to

Table IV. *Previous reports of clinical outcomes in right-sided mCRC patients treated with third-line EGFR inhibitor with or without Irinotecan.*

| Authors | No. of patients | RAS status | Treatment | RR (%) | PFS (months) | OS (months) |
|---|---|---|---|---|---|---|
| Brulé *et al.* | 56 | *KRAS* exon 2 WT | Cetuximab | NA | 1.9 | 6.2 |
| Boeckx *et al.* | 16 | *RAS* WT | Panitumumab | 0 | 1.7 | 4.7 |
| Boeckx *et al.* | 12 | *RAS*/*BRAF* WT | Panitumumab | NA | 1.7 | 6.1 |
| Kim *et al.* | 14 | *RAS*/*BRAF* WT | EGFR inhibitor with or without Irinotecan | NA | 3.7 | 5.7 |
| Loree *et al.* | 18 | *RAS*/*BRAF* WT | Cetuximab | 0 | 3.6 | 5.7 |
| Our data | 31 | *KRAS* exon 2 WT | EGFR inhibitor with or without Irinotecan | 19.4 | 5.3 | 11.8 |
| | 28 | *RAS* WT | EGFR inhibitor with or without Irinotecan | 21.4 | 5.7 | 11.7 |
| | 21 | *RAS*/*BRAF* WT | EGFR inhibitor with or without Irinotecan | 27.3 | 6.5 | 12.1 |

mCRC: Metastatic colorectal cancer; EGFR: epidermal growth factor receptor; *RAS*: rat sarcoma viral oncogene homolog; RR: response rate; PFS: progression-free survival; OS: overall survival; *KRAS*: kirsten rat sarcoma viral oncogene homolog; WT: wild type; NA: not available; *RAS*: rat sarcoma viral oncogene homolog; *BRAF*: raf murine sarcoma viral oncogene homolog B.

Table V. *Previous reports of clinical outcomes in mCRC patients treated with salvage line chemorherapy.*

| Authur | No. of patients | Treatment | *RAS* status | RR (%) | PFS (months) | OS (months) |
|---|---|---|---|---|---|---|
| Jonkeŕ *et al.* | 287 | Cetuximab | All patients | 8.0 | NA | 6.1 |
| Van Cutsem Cutsem *et al.* | 231 | Panitumumab | All patients | 10.0 | 2.0 | NA |
| Cunningham *et al.* | 218 | Cetuximab+Irinotecan | All patients | 22.9 | 4.1 | 8.6 |
| Cunningham *et al.* | 111 | Cetuximab | All patients | 10.8 | 1.5 | 6.9 |
| Price *et al.* | 499 | Panitumumab | *KRAS* exon 2 WT | 22.0 | 4.1 | 10.4 |
| Price *et al.* | 500 | Cetuximab | *KRAS* exon 2 WT | 19.8 | 4.4 | 10.0 |
| Grothey *et al.* | 505 | Regorafenib | All patients | 1.0 | 1.9 | 6.4 |
| Mayer *et al.* | 534 | Trifluridine/tipiracil | All patients | 1.6 | 2.0 | 7.1 |
| Li *et al.* | 136 | Regorafenib | All patients | 3.0 | 3.2 | 8.8 |
| Kuboki *et al.* | 25 | Trifluridine/tipiracil+Bevacizumab | All patients | 4.0 | 5.6 | 11.4 |
| Sakai *et al.* | 61 | Panitumumab+Irinotecan | *KRAS* exon 2 WT | 26.2 | 5.4 | 14.9 |
| Sakai *et al.* | 59 | Cetuximab+Irinotecan | *KRAS* exon 2 WT | 22.0 | 4.3 | 11.5 |
| Pfeiffer *et al.* | 46 | Trifluridine/tipiracil+Bevacizumab | All patients | 2.0 | 4.6 | 9.4 |
| Our data | 72 | EGFR inhibitor with or without Irinotecan | *KRAS* exon 2 WT | 27.8 | 6.1 | 12.7 |
| | 65 | EGFR inhibitor with or without Irinotecan | *RAS* WT | 29.2 | 6.5 | 12.6 |
| | 56 | EGFR inhibitor with or without Irinotecan | *RAS*/*BRAF* WT | 33.3 | 6.8 | 14.0 |

mCRC: Metastatic colorectal cancer; *RAS*: rat sarcoma viral oncogene homolog; RR: response rate; PFS: progression-free survival; OS: overall survival; *KRAS*: kirsten rat sarcoma viral oncogene homolog; WT: wild type; NA: not available; EGFR: epidermal growth factor receptor; *RAS*: rat sarcoma viral oncogene homolog; *BRAF*: raf murine sarcoma viral oncogene homolog B.

examine the other *RAS (KRAS exon 3 ,4 or NRAS)* and *BRAF* status prior to third line treatment in order to maximize the response to EGFR inhibitors.

There are limitations to our study. This was a retrospective study with a relatively small sample size. However, even with these limitations, the results of this study provide important insights into the clinical use of EGFR inhibitors as the third-line treatment, especially in patients with *RAS*/*BRAF* WT right-sided tumors.

In conclusion, our data indicated that the additional analysis of *RAS* and *BRAF* V600E mutations could contribute to the selection of patients who are most likely to benefit from third-line EGFR inhibitors, regardless of primary tumor location.

## Conflicts of Interest

The Authors declare that they have no conflicts of interest in relation to this study.

## Authors' Contributions

Conception and design: TS, HO, ES, AO, and KY; acquisition of data: TS and HO; analysis and interpretation of data: TS, HO, ES, AO; writing, review and/or revision of the manuscript: all Authors; administrative, technical, or material support: TS, HO, ES; study supervision: KY.

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