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FMS-like tyrosine kinase 3 (*FLT3*) amplification in patients with metastatic colorectal cancer

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Abbreviations: CLIA, clinical laboratory improvement amendments; EGFR, epidermal growth factor receptor; FLT3, FMS-like tyrosine kinase 3; mCRC, metastatic colorectal cancer; NGS, next-generation sequencing; OCA, Oncomine™ Comprehensive Assay; OCP, Oncomine™ Cancer Research Panel; OS, Overall survival; PDC, patient-derived tumor cells; PDGFR, platelet-derived growth factor receptor; PFS, progression-free survival; RTK, receptor tyrosine kinase; STAT, signal transducer and activator transcription factor; VEGFR, vascular endothelial growth factor receptor.

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Abstract

FMS-like tyrosine kinase 3 (FLT3) plays a key role in hematopoiesis. However, the oncogenic role of *FLT3* amplification in patients with metastatic colorectal cancer (mCRC) remains unclear. Here, we aimed to evaluate the characteristics, prognosis, and treatment efficacy of an FLT3 inhibitor (regorafenib) in patients with mCRC with *FLT3* amplifications. Tumor tissue samples from 2329 patients were sequenced using NGS in the Nationwide Cancer Genome Screening Project in Japan. The effects of clinicopathological features, co-altered genes, prognosis, and efficacy of regorafenib were investigated. Between April 2015 and June 2018, 85 patients with mCRC with *FLT3* amplification were observed. There were no differences in baseline characteristics between patients with or without *FLT3* amplification. The frequency of *RAS* or other gene co-alterations was inversely correlated with the copy number status. Median survival time in patients with *FLT3* amplification was significantly shorter compared with those with non-*FLT3* amplification. Further investigations of *FLT3* amplification as a potential treatment target in mCRC are warranted.

KEYWORDS
colorectal cancer, copy number status, *FLT3* amplification, next-generation sequencing, prognosis

1 | INTRODUCTION

FMS-like tyrosine kinase 3 (FLT3) is a member of the class III receptor tyrosine kinase family, which includes PDGF-R, KIT, and FMS.[1] The FLT3 protein is normally expressed in hematopoietic progenitor stem cells and plays a key role in controlling the proliferation and differentiation of hematopoietic precursor cells.[2] *FLT3*-activating mutations have been identified in approximately 30% of patients with acute myeloid leukemia.[3] The overexpression of wild-type FLT3 is observed mainly in *MLL*-rearranged acute lymphoblastic leukemia.[4] Overexpression and activating mutations of FLT3 are important targets for molecular therapy and are unfavorable prognostic factors. FLT3 tyrosine kinase inhibitors have been shown to be effective in hematopoietic cancer cells with *FLT3* gene alterations.[5] More recently, comprehensive genomic analyses have indicated that the majority of *FLT3* alterations are somatic mutations, followed by amplification, and *FLT3* alterations have also been observed in solid tumors. The frequency of *FLT3* amplifications was reported to be 1.0% in breast cancer,[6] 3.9% in metastatic colorectal cancer (mCRC),[7] 1.7% in gastric cancer,[8] and 0.9% in lung adenocarcinoma.[9]

Two case reports of patients with mCRC with *FLT3* amplifications, which were identified by targeted genomic profiling, demonstrated the clinical benefit of a multikinase inhibitor with inhibitory activity against FLT3 such as regorafenib or sorafenib.[10,11] Regorafenib is an orally available multikinase inhibitor that showed survival improvement in salvage line treatment in mCRC.[12] It emerged from the process of optimizing sorafenib by modulating its molecular structure,[13] therefore, like sorafenib, regorafenib blocks similar kinases such as Raf serine/threonine kinases (Raf-1, wild-type B-Raf and B-Raf V600E), vascular endothelial growth factor receptor (VEGFR) 1-3, platelet-derived growth factor receptor (PDGFR)-β and FLT3, c-Kit, and RET.[14] In a previous study, Lim et al evaluated the efficacy of regorafenib in mCRC with *FLT3* amplification preclinically and clinically.[11] In their clinical investigation, although, FLT3 expression was slightly reduced following treatment with regorafenib or sorafenib, using PDC from an *FLT3*-amplified colorectal cancer patient, patients with high copy numbers of *FLT3* achieved partial response.[11] Therefore, the association between copy number of *FLT3* and the efficacy of multikinase inhibitors still remains unclear.

To date, the clinical impact and the status of genetic heterogeneity such as co-alteration genes of *FLT3* amplifications in patients with mCRC is yet to be fully evaluated. In this study, we evaluated the characteristics, prognosis, genetic heterogeneity, and treatment efficacy of an FLT3 inhibitor (regorafenib) in patients with *FLT3*-amplified mCRC.

2 | MATERIALS AND METHODS

2.1 | Study design and patients

This was an observational, retrospective, multicenter study on patients with mCRC. Tumor tissue samples from 2329 patients with mCRC were sequenced using the Oncomine Comprehensive Assay, an NGS-based assay, in the Nationwide Cancer Genome Screening Project in Japan (SCRUM-Japan GI-SCREEN). The included patients: (i) had a pathologically confirmed colorectal adenocarcinoma, (ii) received systemic chemotherapy, (iii) had a *RAS* mutational status

identified with a PCR-based assay, (iv) had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0-1, (v) had adequate bone marrow, renal, and hepatic function at the initiation of chemotherapy, (vi) had no other severe medical conditions, and (vii) provided written informed consent. The ethical, medical, and scientific aspects of the study were reviewed and approved by the Institutional Review Board. This trial was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000016343). The study was conducted in accordance with the 1975 Declaration of Helsinki, revised in 2000.

2.2 | Targeted sequencing

Patients' biopsies or archived surgically resected samples were sent to a CLIA-certified clinical laboratory in the United States. In the CLIA laboratory, tumor DNA and RNA were extracted and used for NGS-based amplicon sequencing with the Oncomine™ Cancer Research Panel (OCP) or Ion Torrent™ Oncomine™ Comprehensive Assay (OCA; Thermo-Fisher Scientific, Waltham, MA). These assays covered 143 (v1, 2015-2017) and 161 (v3, 2017 to current day) of the most relevant cancer-related genes, respectively, and detected relevant single nucleotide variants, copy number variations, gene fusions, and indels in 1 streamlined workflow. The annotated genome variant call format files and the binary version of the sequence alignment/map files were stored at the SCRUM-Japan Data Center.

2.3 | Copy number status of *FLT3* and other genes using tissue NGS

FLT3 gene copy number alteration was evaluated (Figure S1), and *FLT3* amplification and other genes were defined as copy number ≥ 7.0 by OCA/OCP, which has been analytically and clinically validated.[15,16] *FLT3* amplification status was divided into 2 categories using the median copy number. High amplification was defined as copy number ≥ 10.0, and low amplification was defined as 7 ≤ copy number < 10.0. A mutation was identified if the allele frequency was more than 5% and the depth of coverage was more than 250.

We evaluated the frequency of co-alteration status of potential driver genes such as *RAS/ BRAFV600E* and other genes related to RTKs or those located on chromosome 13 in *FLT3*-amplified mCRC patients. In addition, we evaluated the prognosis in patients with *FLT3* amplifications who received at least second-line or more chemotherapy, which is widely accepted as the standard treatment for mCRC.

2.4 | Statistical analyses

OS and PFS were calculated using the Kaplan-Meier method and compared with the log-rank test. During systemic chemotherapy, which included treatment with regorafenib, each patient was assessed for objective response to treatment in accordance with the Response Evaluation Criteria in Solid Tumors (v.1.1) with computed tomographic scans performed every 2-3 mo until disease progression. The disease control rates represented the percentage of patients with a complete response, partial response, and stable disease. Differences in proportion were evaluated with Pearson chi-square test. The significance of differences in age was estimated with Kruskal-Wallis test. Statistical analyses were conducted using SAS 9.3 software (SAS Institute, Cary, NC). All tests were 2-sided, and a P-value < .05 was considered to indicate statistical significance.

3 | RESULTS

3.1 | Patient characteristics

Between April 2015 and June 2018, 2078 patients who met the study inclusion criteria were recruited (Figure 1). Of these, 85 patients (3.6%) with mCRC and who had *FLT3* amplifications were analyzed. There were no significant differences in baseline characteristics between patients with *FLT3* amplifications (high and low) and those with non-*FLT3* amplifications, as shown in Table 1. The OCP or OCA results demonstrated that enrichment of *TP53* mutations showed a trend for higher frequency in *FLT3* amplification mCRC patients compared with in non-*FLT3* amplification patients, but the difference did not reach statistical significance (P = .16). The frequency of *RAS* mutations was similar in these 3 cohorts (P = .12). In contrast, there were activating alterations in both *BRAFV600E* (2.5% in low amplification vs 0% in high amplification vs 6.2%, P = .03) and there was a trend toward a lower frequency of *PIK3CA* mutations in patients with high *FLT3* amplification compared with those with low or non-*FLT3* amplification (7.3% in non-amplification and 10% in low amplification vs 2.2% in high amplification, P = .14).

3.2 | Correlation between *FLT3* amplification status and clinicopathological features, including the status of other gene alterations

We evaluated clinicopathological variables according to *FLT3* amplification status to investigate the clinical relevance of copy numbers of *FLT3* amplifications. *FLT3* amplification status was not associated with age or pathological grading, whereas the status of high *FLT3* amplification was more frequently observed in females and in the rectum compared with that of low *FLT3* amplification (Table 1). The frequency of *RAS* mutations was significantly lower in patients with high *FLT3* amplification compared with in patients with low *FLT3* amplification (37.8% vs 60.0%, P = .04), although the frequency of *TP53* was similar regardless of *FLT3* amplification status (Figure 2A,B). With regards to the co-amplification status related to RTKs such as *ERBB2*, *EGFR*, *FGFR*, and

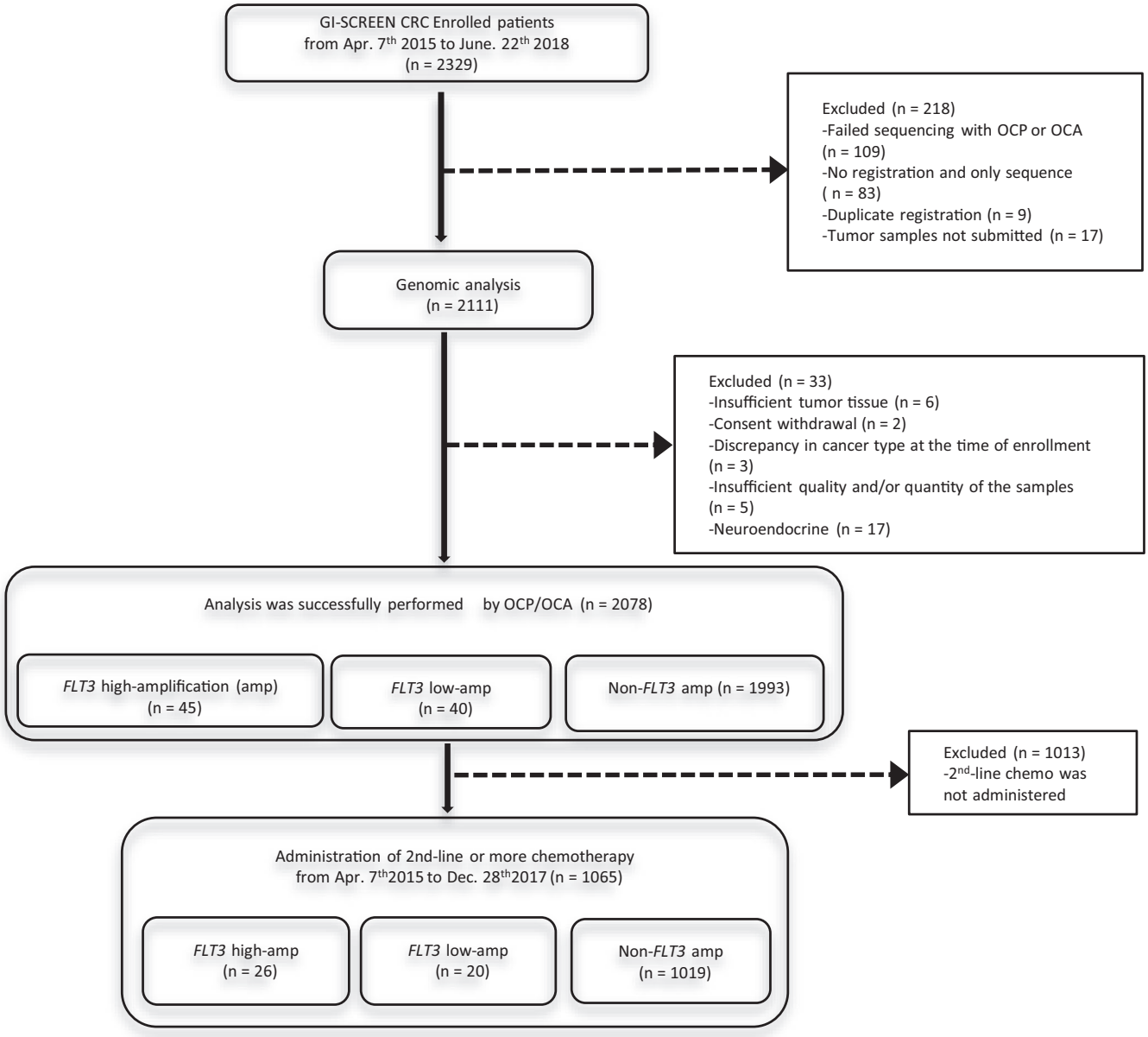

FIGURE 1 Flow diagram of this study. amp, amplification

MET, concurrent amplification was more common in low *FLT3* amplification (Figure 2A). To assess the co-amplification genes on the long arm of chromosome 13 at position 12 where *FLT3* is located, we evaluated amplifications in *BRCA2*, which is also located on the long arm of chromosome 13 at position 13.1 and *GAS6*, which is located on the long arm of chromosome 13 at position 34, in addition to *RAS*, *BRAF*, and *ERBB2*, which are known oncogenic driver genes in mCRC patients (Figure 3A,B). More than half of the patients with low *FLT3* amplification had co-mutations in *RAS* and 40% of these patients had co-amplification of *BRCA2* or *GAS6* on chromosome 13 (Figure 3A). In contrast, more than half of patients with high *FLT3* amplification had no co-occurring alterations (Figure 3B). The frequency of co-alterations was significantly lower in patients with high *FLT3* amplification compared with those with low *FLT3* amplification (75% vs 38%, $P < .001$).

3.3 | Prognostic significance of *FLT3* amplification status

To determine the prognostic significance of *FLT3* amplification, we performed survival analyses for 1065 patients who received at least second-line or more chemotherapy. The mean follow-up time was 24 mo (range: 6-127 mo), and 451 patients (45.3%) died. There were no significant differences in patient characteristics and treatment regimens between the cohorts (Table S1). The median OS from first-line chemotherapy was significantly shorter in patients with low or high amplification compared with those without *FLT3* amplification (29.5 mo in low amplification and 24.5 mo in high amplification vs 43.6 mo without *FLT3* amplification, $P = .003$ and $P = .002$, respectively; Figure 4). In comparison between patients with high and low *FLT3* amplification, the median OS in patients with low amplification

TABLE 1 Patients' characteristics

	Non-*FLT3* amp (n = 1993)	*FLT3* amp (n = 85)			
		Low amp (n = 40, 1.9%) n (%)	High amp (n = 45, 2.2%) n (%)	P^a	P^b
Age					
Median, (range) (y)	64 (21-88)	65 (35-84)	65 (32-83)	.85	.88
Gender					
Male	1103 (55.3)	29 (72.5)	21 (46.7)	.56	.02
Female	90 (44.7)	11 (28.5)	24 (54.3)		
Primary site					
Right colon	619 (31.1)	11 (27.5)	13 (28.9)	<.01	.06
Left colon	718 (36.0)	15 (37.5)	12 (71.1)		
Rectum	628 (31.5)	10 (25.0)	20 (44.4)		
Unknown	28 (1.4)	4 (10.0)	0 (0.0)		
Histology (grade)					
Grade 1	543 (27.2)	8 (20.0)	9 (20.0)	.20	.75
Grade 2	1201 (60.3)	26 (65.0)	33 (73.3)		
Grade 3	94 (4.7)	3 (7.5)	1 (2.2)		
Others	23 (5.2)	2 (5.0)	1 (2.2)		
Unknown	52 (2.6)	1 (2.5)	1 (2.2)		
RAS					
Wild-type	1149 (57.2)	16 (40.0)	28 (63.2)	.12	.04
Mutant	844 (42.8)	24 (60.0)	17 (37.8)		
BRAF V600E					
Wild-type	1869 (93.8)	39 (97.5)	45 (100.0)	.03	.29
Mutant	124 (6.2)	1 (2.5)	0 (0.0)		
PIK3CA					
Wild-type	1763 (88.4)	36 (90.0)	44 (97.8)	.14	
Mutant	230 (7.3)	4 (10.0)	1 (2.2)		.13
ERBB2					
Non-Amp	1938 (97.2)	39 (97.5)	45 (93.3)	.37	.29
Amp (CN ≥ 7.0)	55 (2.8)	1 (5.0)	0 (0.0)		
TP53					
Wild-type	711 (35.7)	11 (27.5)	11 (24.5)	.16	.75
Mutant	1282 (64.3)	29 (72.5)	34 (75.5)		
BRCA1					
Wild-type	1859 (93.2)	31 (77.5)	38 (80.0)	<.01	.41
Mutant	134 (6.8)	9 (22.5)	7 (15.6)		
BRCA2					
Wild-type	1731 (86.9)	30 (75.0)	44 (97.8)	<.01	<.01
Mutant	262 (13.1)	0 (0.0)	0 (0.0)		
Amp (CN ≥ 7.0)	0 (0.0)	10 (25.0)	1 (2.2)		

Abbreviations: amp, amplification; CN, copy number.

[a]*P*-values were calculated including 3 cohorts (Non-*FLT3* amp and *FLT3* amp: Low amp and high amp) using Pearson chi-square test.

[b]*P*-values were calculated between 2 cohorts (Low amp and high amp) by Pearson chi-square test.

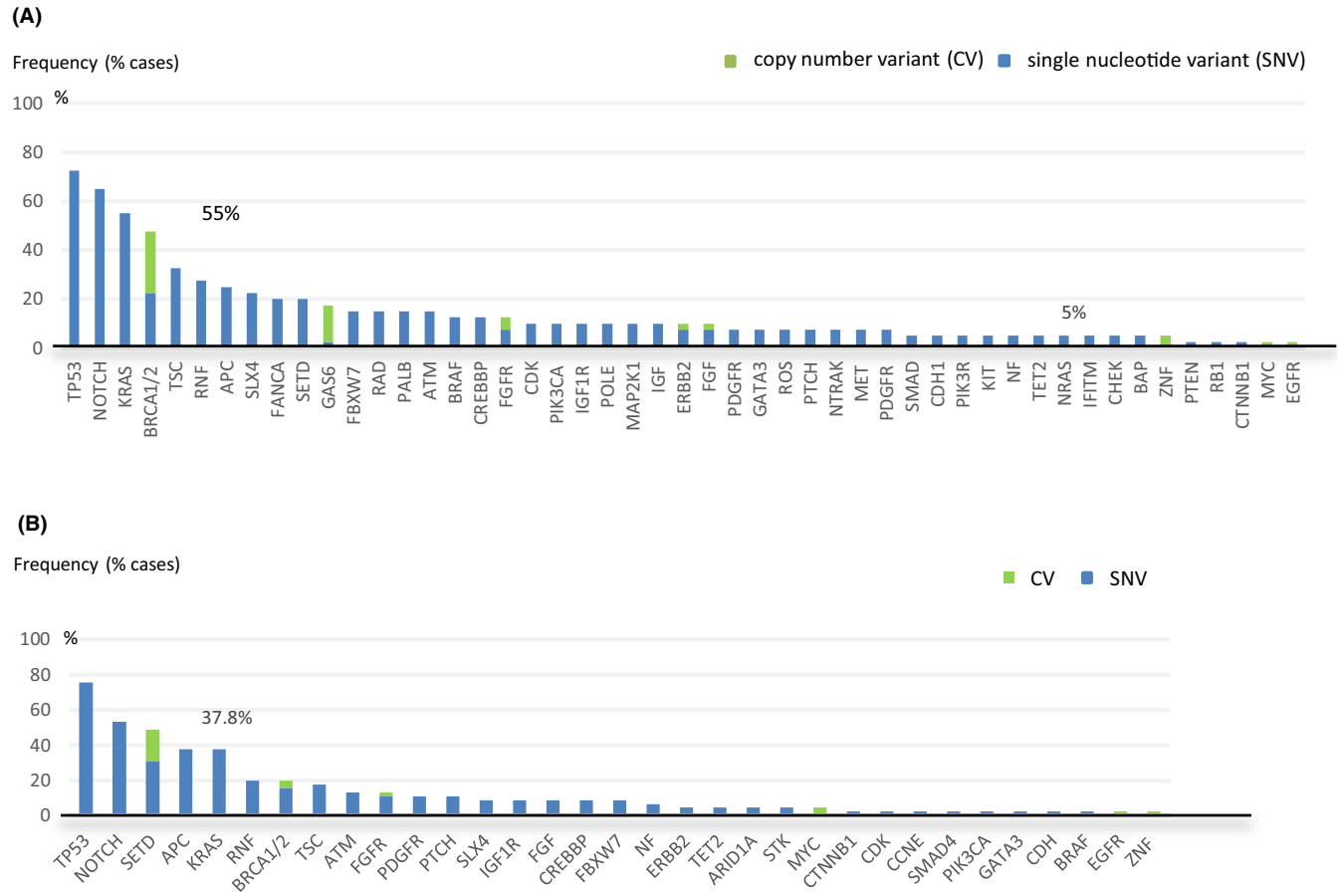

FIGURE 2 Long tail plots for FLT3 amplified metastatic colorectal cancer. A, Concurrent genomic alterations in FLT3 low-amplified cases (n = 40). B, Concurrent genomic alterations in FLT3 high-amplified cases (n = 45)

tended to be better compared with that in patients with high amplification (29.5 mo in low amplification vs 24.5 mo in high amplification, $P = .12$). Furthermore, among patients with low *FLT3* amplification, 13 of 20 (65.0%) patients had *RAS* mutation and 1 (5%) patient had *ERBB2* amplification (Table S1), while the others (n = 6) did not.

3.4 | Treatment efficacy of regorafenib according to *FLT3* gene copy number status

The treatment efficacy of regorafenib was evaluated in 20 patients for whom the data of tumor responses to regorafenib were available among all patients whose gene analysis was successfully performed (n = 2078) (Table 2). The disease control rate was numerically but not statistically higher in patients with *FLT3* amplification compared with in those with non-*FLT3* amplification, as shown in Table 2 (57.1% vs. 23.0%).

4 | DISCUSSION

This study aimed to identify the clinical significance and status of genetic heterogeneity in patients with *FLT3*-amplified mCRC. Here, we observed *FLT3* amplifications in approximately 4% of patients including cases with high copy number of *FLT3*. In the current study, *FLT3* amplifications were identified more commonly in left side colon compared with in right side colon, and the amplification status was associated with co-alteration gene status of other driver genes and other genes on chromosome 13. Furthermore, the copy number status was associated with prognosis, and the efficacy of regorafenib. To the best of our knowledge, this is the first study to evaluate the characteristics and status of genetic heterogeneity according to copy number status of *FLT3* using NGS in a large-scale study of *FLT3*-amplified mCRC.

To date, the status of concurrent alterations in *FLT3*-amplified mCRC remains unclear. In our study, *c.* 50% of the patients with *FLT3* amplifications also harbored *RAS* mutations, which are common driver genes for mCRC,[17] although other driver genes, such as *BRAF* and *PIK3CA*, were rarely co-mutated with the *FLT3* gene. With regards to patients with *FLT3* amplification status, the frequency of co-occurring *RAS* mutation was higher in patients with low *FLT3* amplification compared with in those with high *FLT3* amplification. This frequency was inversely correlated with *FLT3* gene copy number. These findings suggested that the status of high *FLT3* amplifications may function as an oncogenic driver alteration that promotes the proliferation of cancer cells, similar to a *RAS* mutation. Regarding

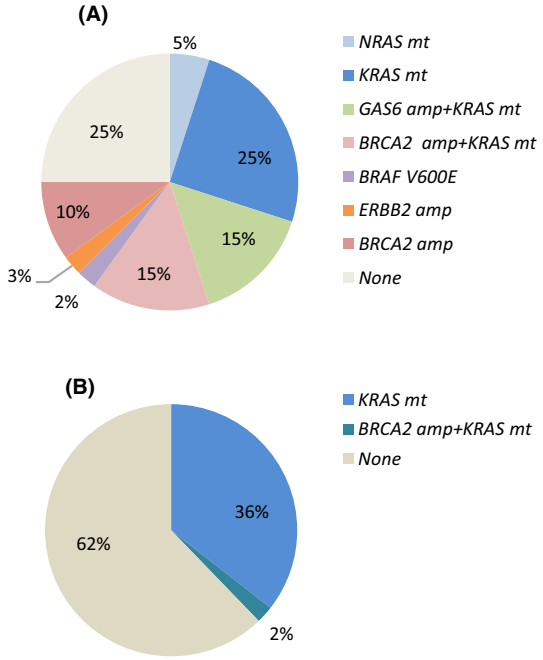

FIGURE 3 Overlapping alterations on chromosome 13 and other potential driver genes in FLT3 amplified metastatic colorectal cancer. A, Overlapping alterations in FLT3 low-amplified cases (n = 40). B, Overlapping alterations in FLT3 high-amplified cases (n = 45). amp, amplification; mt, mutation

the co-amplification gene status on chromosome 13 where the *FLT3* gene is located and other co-altered oncogenic driver genes, the frequency of co-alteration genes was higher in patients with low *FLT3* amplification compared with those with high *FLT3* amplification. The co-occurring alteration status of other genes was inversely associated with the copy number status of the *FLT3* gene. In hematopoietic cells, FLT3 induces the activation of signal transduction networks mainly through PI3K and the RAS cascade, supporting the activation of AKT, signal transducer and activator transcription factor (STAT), and extracellular-signal regulated kinases (ERK) 1 and 2.[18] Therefore, the presence of co-amplified and co-altered genes is implicated in innate and acquired resistance to receptor tyrosine kinase-targeted therapies for *FLT3*.

TABLE 2 Treatment efficacy of regorafenib in mCRC patients

FLT3 amp status	n	CR + PR	SD	PD	DCR
Non-amplification	13	0	3	10	23.0%
Amplification	7	0	4	3	57.1%

Abbreviations: amp, amplification; CR, complete remission; DCR, disease control rate; PD, progressive disease; PR, partial response; SD, stable disease.

Two types of amplification patterns have been reported in *ERBB2* or *MET* genes, ie, focal and non-focal amplifications, in which the gene copy number gain was due to chromosomal aneuploidy. Among patients with increased *MET* copy numbers, polysomy for chromosome 7 (the chromosome on which *MET* is located) was observed in *c*. 30% of patients with non–small-cell lung cancer[19] and gastric cancer.[20] Furthermore, it was reported that non-focal *MET* amplification did not act as an oncogenic driver.[20] Therefore, low copy numbers of the *FLT3* gene, regarded as non-focal amplification, may not function as an oncogenic driver, unlike high amplification of the *FLT3* gene. Indeed, patients with high *FLT3* amplification had less driver co-alteration genes, while 14 of 20 (70.0%) patients with low *FLT3* amplification had some driver co-alteration genes such as *RAS* or *ERBB2* (Table S1), associated with poor prognosis. The trend of shorter OS in patients with high FLT3 amplification, compared with those with low *FLT3* amplification, suggested that high *FLT3* amplification may be associated with the tumor biology and be a potential treatment target, similar to *ERBB2* amplification in mCRC.[21]

As for the efficacy of regorafenib in our study, disease control rate in patients with *FLT3* amplification tended to be slightly better compared with that in patients with no amplification. In the current study

FLT3 amp status	N	Median OS (months)	*P*	HR	95% CI	*P*	HR	95% CI
Non-amp	1019	43.6	Ref	Ref	-	-	-	-
Low-amp	20	29.5	0.003	1.82	1.10-3.00	Ref	Ref	-
High-amp	26	24.5	0.002	2.68	1.47-4.92	0.12	1.87	0.82-4.48

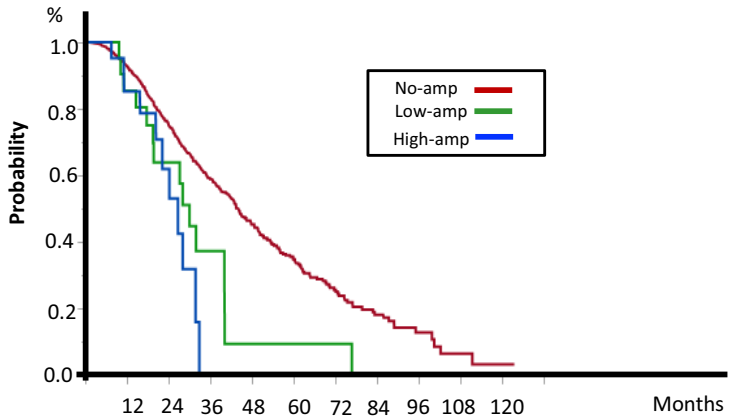

FIGURE 4 Overall survival according to FLT3 amplification status. Kaplan-Meier curve for patients with high FLT3 amplification (n = 26, blue) and low FLT3 amplification (n = 20, green), and those without FLT3 amplification (n = 1019, red). amp, amplification; CI, confidence interval; HR, hazard ratio; OS, overall survival

and another previous report,[11] regorafenib, which is a mild inhibitor of FLT3 with an IC_{50} of 162 nmol/L,[14] showed moderate activity in patients with *FLT3* amplifications. Moreover, previous case reports have reported that patients with metastatic colon cancer harboring *FLT3* amplifications, determined by targeted sequencing, exhibited partial responses when heavily treated with regorafenib or sorafenib,[10,11] which partially blocked the activity of FLT3. Conversely, the recent TAPUR trial investigated the efficacy of sunitinib administration in patients with mCRC with *FLT3* amplification.[22] However, monotherapy with sunitinib did not demonstrate sufficient activity in these patients. This may have been due to the inhibition of phosphorylation of wild-type FLT3 by sunitinib with an IC_{50} of 250 nmol/L in vitro.[23] In contrast, a previous case report showed promising efficacy for sorafenib with an IC_{50} of 58 nmol/L in wild-type FLT3.[10,24] Given the limited efficacy of regorafenib with a disease control rate of 40% in mCRC,[25] a therapeutic strategy with specific inhibition of FLT3 needs to be explored for *FLT3*-amplified mCRC in the future.

Additionally, Siravegna et al,[26] reported that patients with *FLT3* amplifications may be resistant to anti-EGFR therapy. In their report, 2 of 10 patients with mCRC with primary resistance to anti-EGFR therapy harbored *FLT3* amplifications. In our study, it was difficult to evaluate the presence of primary resistance because there were few patients who had received anti-EGFR therapy as first-line treatment. Further evaluation of resistance to anti-EGFR therapy is also needed for patients with *FLT3* amplifications.

Our study has several limitations. We did not assess FLT3 expression immunohistochemically or conduct basic research to clarify the molecular mechanisms of the antitumor activity of the multikinase inhibitors of *FLT3* amplification. As for the efficacy of regorafenib, in our study, it was difficult to evaluate accurately whether this drug specifically inhibited the activation of FLT3 in *FLT3*-amplified mCRC in terms of spectrum of kinase inhibitory profile and in a small number of patients. Large-scale studies and further basic research are required to validate our results.

In conclusion, 3.6% of patients with mCRC exhibited *FLT3* amplifications. The copy number status was inversely related to the frequency of co-existing alterations, and prognosis. High *FLT3* amplification most commonly existed without other alterations, indicating focal amplification. The association between copy number status of *FLT3* and clinical outcome requires further investigations to clarify whether it could be a promising target for future cancer treatment in mCRC.

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CONFLICT OF INTEREST

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REFERENCES

1. Blume-Jensen P, Hunter T. Oncogenic kinase signaling. *Nature*. 2001;411:55-365.
2. Mackarehtschian K, Hardin JD, Moore KA, Boast S, Goff SP, Lemischka IR. Targeted disruption of the flk2/flt3 gene leads to deficiencies in primitive hematopoietic progenitors. *Immunity*. 1995;3:147-161.
3. Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. *Blood*. 2002;100:1532-1542.
4. Armstrong SA, Kung AL, Mabon ME, et al. Inhibition of FLT3 in MLL. Validation of a therapeutic target identified by gene expression based classification. *Cancer Cell*. 2003;3:173-183.
5. Williams B, Atkins A, Zhang H, et al. Cell-based selection of internalizing fully human antagonistic antibodies directed against FLT3 for suppression of leukemia cell growth. *Leukemia*. 2005;19:1432-1438.
6. Network CGA. Comprehensive molecular portraits of human breast tumors. *Nature*. 2012;490:61-70.
7. Cancer Genome Atlas Network: Comprehensive molecular characterization of human colon and rectal cancer. *Nature*. 2012;487:330-337.
8. Cancer Genome Atlas Network: Comprehensive molecular characterization of gastric adenocarcinoma. *Nature*. 2014;513:202-209.
9. Cancer Genome Atlas Network: Comprehensive molecular characterization of lung adenocarcinoma. *Nature*. 2014;511:543-550.
10. Moreira RB, Peixoto RD, de Sousa Cruz MR. Clinical Response to Sorafenib in a patient with metastatic colorectal cancer and FLT3 amplification. *Case Rep Oncol*. 2015;8:83-87.
11. Lim SH, Kim SY, Kim K, et al. The implication of FLT3 amplification for FLT targeted therapeutics in solid tumors. *Oncotarget*. 2017;10:3237-3245.
12. Grothey A, Van Cutsem E, Sobrero A, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet*. 2013;381:303-312.
13. de la Fouchardière C. Regorafenib in the treatment of metastatic colorectal cancer. *Future Oncol*. 2018;14:2239-2246.
14. Wilhelm SM, Dumas J, Adnane L, et al. Regorafenib (BAY 73–4506): a new oral multikinase inhibitor of angiogenic, stromal and

oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. *Int J Cancer*. 2011;129:245-255.
15. Yuki A, Chitwood J, Sidhu H, et al. Analytical validation of the oncomine comprehensive assay v3 with FFPE and cell line tumor specimens in a CAP-accredited and CLIA-certified clinical laboratory. *J Mol Diagn*. 2018;20:972, ST016.
16. Lih CJ, Harrington RD, Sims DJ, et al. Analytical validation of the next-generation sequencing assay for a nationwide signal-finding clinical trial: molecular analysis for therapy choice clinical trial. *J Mol Diagn*. 2017;19:313-327.
17. Janakiraman M, Vakiani E, Zeng Z, et al. Genomic and biological characterization of exon 4 KRAS mutations in human cancer. *Cancer Res*. 2010;70:5901-5911.
18. Scholl C, Gilliland DG, Frohling S. Deregulation of signaling pathways in acute myeloid leukemia. *Semin Oncol*. 2008;35:336-345.
19. Go H, Jeon YK, Park HJ, Sung SW, Seo JW, Chung DH. High Met gene copy number leads to shorter survival in patients with non-small cell lung cancer. *J Thorac Oncol*. 2010;5:305-313.
20. Janjigian YY, Tang LH, Coit DG, et al. Met expression and amplification in patients with localized gastric cancer. *Cancer Epidemiol Biomarkers Prev*. 2011;20:1021-1027.
21. Sawada K, Nakamura Y, Yamanaka T, et al. Prognostic and predictive value of HER2 amplification in patients with metastatic colorectal cancer. *Clin Colorectal Cancer*. 2018;17:198-205.
22. Alvarez RH, Garrett-Mayer E, Halabi S, et al. Sunitinib (S) in patients (Pts) with metastatic colorectal cancer (mCRC) with *FLT-3* alterations: Results from the Targeted Agent and Profiling Utilization Registry (TAPUR) Study. *Proc Am Assoc Cancer Res Annu Meet USA*. 2019;79(13):CT146.
23. O'Farrell AM, Abrams TJ, Yuen HA, et al. SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity in vitro and in vivo. *Blood*. 2003;101:3597-3605.
24. Wilhelm SM, Carter C, Tang L, et al. BAY 43–9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res*. 2004;64:7099-7109.
25. Grothey A, Van Cutsem E, Sobrero A, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet*. 2013;381:303-312.
26. Siravegna G, Mussolin B, Buscarino M, et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. *Nat Med*. 2015;21:795-801.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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