Clinical and molecular distinctions in patients with refractory colon cancer who benefit from regorafenib treatment

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***Abstract*:** Regorafenib (Stivarga, BAY 73-4506; Bayer Pharma AG, Berlin, Germany) is a novel oral multikinase inhibitor that blocks the activity of several protein kinases. However, few guidelines exist for novel biomarkers to select patients who will likely benefit from regorafenib treatment. Metastatic colorectal cancer (mCRC) patients treated with regorafenib were evaluated in this study. Tumor tissues of these patients were subjected to next-generation sequencing-based cancer panel tests. The relationship between molecular profiling and efficacy of regorafenib was analyzed. Among the 76 mCRC patients, the median age was 58 years (range 22–79 years), and 73.7% received regorafenib as a third-line therapy. The primary tumor locations were the right side ($n = 15$, 19.8%) and the left side ($n = 61$, 80.2%). Most patients (97.4%) had received prior anti-angiogenetic agents, and a prior anti-Epidermal Growth Factor Receptor (EGFR) agent had been administered to 32.9%. Of these 76 patients, 65 were evaluated to determine the efficacy of treatment. We observed zero complete responses, seven confirmed partial responses (PR 9.2%), 26 stable disease states (34.2%), and 32 disease progressions (42.1%). The overall confirmed response rate and the disease control rate were 9.2% and 43.4%, respectively. Genomic analysis revealed that APC mutations were significant in patients who demonstrated a tumor response to regorafenib ($p < 0.05$). Interestingly, FGFR1 amplification was detected in only three of 76 patients (3.9%), and these three patients achieved a PR to regorafenib. The median progression-free survival time was 2.8 months (95% Confidence Interval [CI] 1.6–4.0). Patients with BRAF mutation and/or SMAD4 mutation had significantly worse progression-free survival (PFS) than those without such a mutation. On pathway analysis, Tumor Growth Factor (TGF)-beta pathways were significantly associated with worse PFS. We found that efficacy of regorafenib might be correlated with specific genetic aberrations, such as APC mutation and FGFR1 amplification. In addition, SMAD4 mutation and TGF-beta pathway were associated with worse PFS after regorafenib. We found that efficacy of regorafenib might be correlated with specific genetic aberrations, such as APC mutation and FGFR1 amplification. In addition, SMAD4 mutation and the TGF-beta pathway were associated with worse PFS after regorafenib.

Keywords: colorectal cancer (CRC), next-generation sequencing (NGS), pathway, regorafenib

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Introduction

The prevalence of colorectal cancer (CRC) and the mortality of metastatic colorectal cancer (mCRC) are increasing worldwide.[1] The current treatment for mCRC is systemic chemotherapy, including 5-fluoropyrimidines (5FU), oxaliplatin, irinotecan, and molecularly targeted agents such as anti-Vascular Endothelial Growth Factor Receptor (VEGFR) and anti-Epidermal Growth Factor Receptor (EGFR) inhibitors.[2–4] However, many patients experience disease progression after treatment with available chemotherapies. For those patients, regorafenib has been regarded as the next step of standard therapy.[5,6] Regorafenib's

role as a multi-targeting kinase inhibitor with a broad range of therapeutic targets includes kinases involved in regulation of tumor angiogenesis [VEGFR1 (also known as FLT1), VEGFR2 (KDR), VEGFR3 (FLT4), TIE2 (TEK)], oncogenesis (KIT, RET, RAF1, BRAF, and BRAFV600E), and the tumor microenvironment (PDGFR and FGFR).[7]

Careful patient selection for specific treatments is challenging in the current oncology era. The identification and confirmation of relevant predictive markers to specific agents have improved patient survival and protected them from treatment-related toxicities.[8] However, no promising biomarkers to regorafenib have been identified.[6,9,10] Previous preclinical and clinical studies have proposed candidate biomarkers to predict the anti-tumor activity of regorafenib,[11–13] but those biomarkers have not been sufficient for clinical practice. Recently, new approaches to find novel biomarkers to regorafenib have been tried.[9,14,15]

Due to the increased efficiency of next-generation sequencing (NGS), deep targeted sequencing panels with high depth and high exon coverage are rapidly being developed and used in clinical fields.[16,17] Herein, we evaluated novel biomarkers, including clinicopathological and molecular values, to predict the outcomes of regorafenib in patients with refractory mCRC using NGS testing of tumor tissues.

Patients and methods

Patients

mCRC patients who received regorafenib monotherapy at Samsung Medical Center between January 2018 and January 2019 were included in this analysis. All patients had previously received fluorouracil, irinotecan, and oxaliplatin with or without biological agents such as cetuximab or bevacizumab/aflibercept. All patients were tested using the same NGS platform, the oncomine comprehensive assay (OCA; a commercial test consisting of 143 actionable genes), before starting regorafenib treatment. Each patient's medical records, which included age, sex, primary tumor site, histological type, extent of metastasis, treatment details, and treatment outcomes, were analyzed. We also evaluated patient data on treatment outcomes with regorafenib. This study was approved by the Institutional Review Board of Samsung Medical Center (SMC-IRB #2020-07-032-001) and was conducted in accordance with the ethical principles of the Declaration of Helsinki and the Korea Good Clinical Practice guidelines. All patients provided written informed consent and written informed consent included the disclosure of information, competency of patients to make a decision, and voluntary nature of decision for the purpose, benefit and potential risk of this study.

Next-generation sequencing test

NGS was performed on formalin-fixed, paraffin-embedded specimens using an extensively validated platform (Oncomine Comprehensive Assay v1; ThermoFisher Scientific, Waltham, MA, USA; www.thermofisher.com). The methods for DNA/RNA extraction and sequencing/reporting/validation of the assay were carried out according to previously published reports. For two patients, the genomic profiles were identified using RNA, so their genomic data were excluded from further analysis.

Pathway analysis

Genes with single-nucleotide variations, insertions/deletions, and/or copy number variations were clustered according to the associated oncogenic pathway. If a sample possessed any genetic alterations in a specific pathway, the sample was considered to be the altered case in the pathway.

Statistical analysis

Descriptive statistics were applied to summarize patient characteristics. Response categories were assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. Each nominal variable was compared using Fisher's exact test or the χ^2 test. Progression-free survival (PFS) was defined as the time from starting regorafenib to documentation of disease progression or death. The PFS was estimated using the Kaplan–Meier method along with log-rank analysis. Two-sided null hypotheses of no difference were rejected if the p-values were < 0.05, or if the 95% confidence intervals (CIs) of risk point estimates were excluded. Cox proportional hazards regression modeling was employed in univariate analysis to identify the significant and independent prognostic factors for various clinical parameters and molecular aberrations for survival. All analyses to evaluate the association between genetic alterations and responses to regorafenib were performed using R,

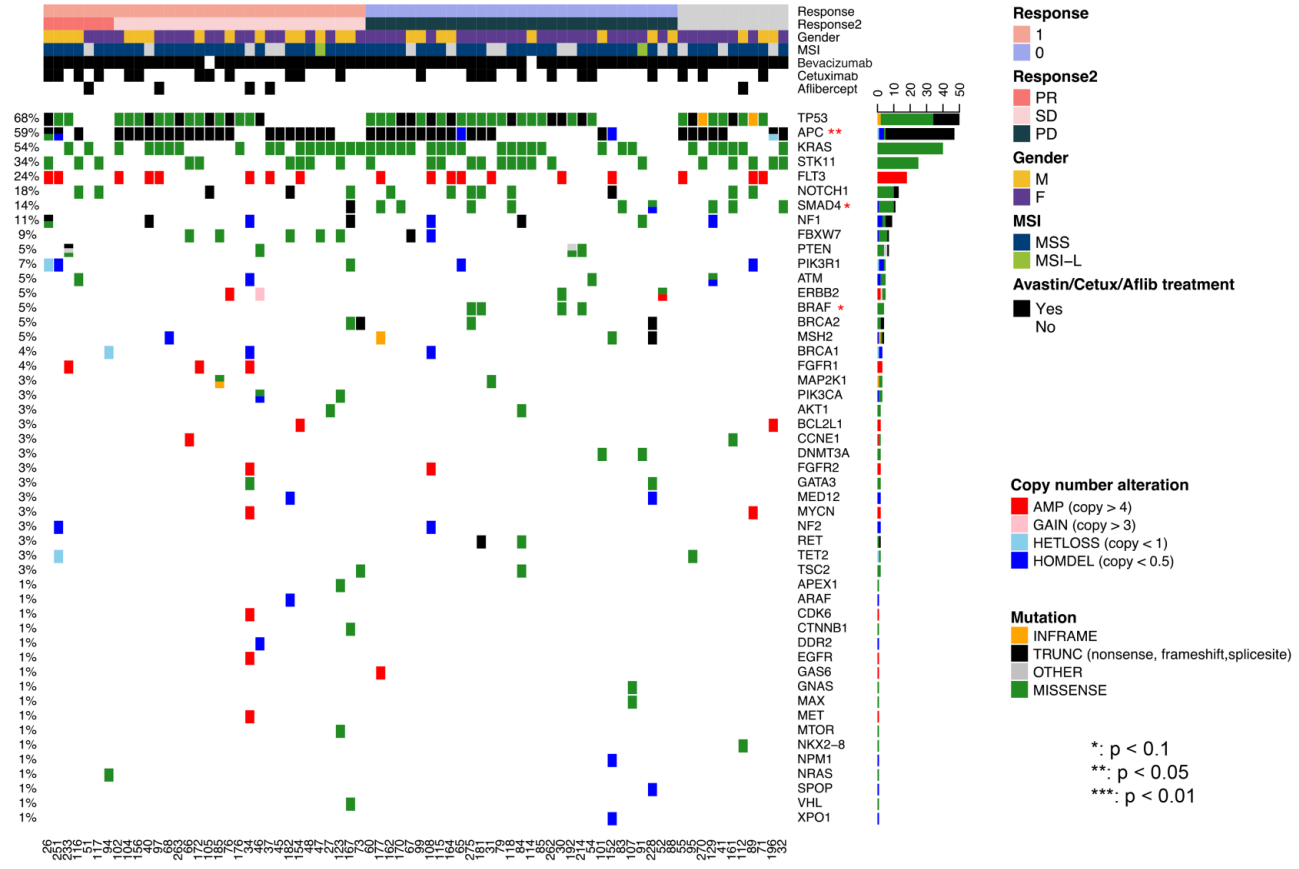

Figure 1. The genomic landscape of the patients. Types of somatic mutations involving specific genes, including significantly mutated genes by mutation frequency and found by NGS. The mutation rates of each gene were marked on the left in percentage. NGS, next-generation sequencing. Fisher's exact test, ***$p < 0.01$; **$p < 0.05$; *$p < 0.5$; the grey star indicates a significantly different constitution of mutation types.

and other analyses were carried out using the Statistical Package for the Social Sciences (SPSS), version 19.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

Seventy-six patients were included in this study between January 2018 and November 2019. The baseline characteristics are presented in Table 1. The median age was 58 years (range 22–79), and the numbers of women and men were 48 (63.2%) and 28 (36.8%), respectively. The median Eastern Cooperative Oncology Group performance status score was 1. Most pathological differentiation results were of good or moderate type (80.2%), and the primary locations of tumors were the left side (80.2%) and the right side (19.8%), respectively. Seventy-four (97.4%) patients had received prior anti-angiogenetic agents such as bevacizumab and/or aflibercept, and 25 (32.9%) were previously treated with cetuximab-containing therapies.

Genomic landscape and pathway analysis of the study population

The genomic landscape of the patients is shown in Figure 1. TP53 mutations (68%) were the most frequently reported. Mutations in APC (57%), KRAS (54%), and STK11 (34%) were identified with a high frequency. In addition, there were alterations in SMAD4 (14%), NF1 (11%), and BRAF (5%). Amplifications in FLT3 (24%), FGFR1 (4%), ERBB2 (3%), FGFR2 (3%), and MYCN (3%) were also observed. Figure 2 shows the distribution of signaling pathways involved in patients. RAS-MAPK signaling (70%), TP53 signaling (69%), and WNT signaling (61%) were commonly involved in these tumors. Signaling analysis revealed that multiple signal pathways coexisted within one tumor in the same patient.

Table 1. Patient characteristics.

Characteristics	Patients ($n=76$)	Patients (%)
Age, years		
⩽65	60	78.9
65<	16	21.1
Sex		
male	28	36.8
female	48	63.2
ECOG		
0	9	11.8
1	67	88.2
Pathology		
Good/moderate	61	80.2
Poor/mucinous	15	19.8
Site of primary tumor		
Right side	15	19.8
Left side	61	80.2
RAS mutation		
KRAS mutation	40	52.6
NRAS mutation	1	1.3
No. of metastasis sites		
⩽2	64	84.2
3⩽	12	15.8
No. of prior chemotherapy rounds		
⩽2	52	73.7
3⩽	24	31.6
Use of prior anti-angiogenesis agents	74	97.4
Use of prior anti-EGFR agents	25	32.9
Duration from the initiation of 1st line chetotherapy to the time of starting regorafenib		
⩽20 months	33	43.4
20 months<	43	56.6

ECOG, Eastern Cooperative Oncology Group.

Efficacy of regorafenib

Among the 76 total patients, 65 were evaluated to determine the efficacy of treatment. We observed no complete responses, seven confirmed partial responses (PR 9.2%), 26 stable disease states (34.2%), and 32 disease progressions (42.1%) (Table 2). The overall confirmed response rate and disease control rate were 9.2% and 43.4%, respectively. Genomic analysis (Figure 1) revealed that APC mutations were significantly associated with response to regorafenib ($p=0.04$). Interestingly, FGFR1 amplification was detected in only three of 76 patients (3.9%), and these three patients achieved a PR to regorafenib. No particular signal pathway was related to the efficacy of regorafenib (Figure 2).

Analysis of the prognostic biomarkers that influence PFS with regorafenib

We conducted an analysis to identify any prognostic values that may affect PFS using Cox proportional hazards regression modeling (see Supplemental Figure 1). Based on our analysis, mutations in BRAF (PFS, wild type; 85.0 days *versus* mutant type; 41.5 days, $p=0.04$) and SMAD4 (PFS, wild type; 90.0 days *versus* mutant type; 51.0 days, $p=0.01$) were significant prognostic factors for a worse PFS to regorafenib (Figures 3A–C). In terms of signaling pathways, patients with a TGF-beta pathway showed a significantly poor PFS to regorafenib compared to those without a TGF-beta pathway (Figure 3D).

Discussion

In this study, we demonstrated that APC mutation was significantly associated with response to regorafenib ($p<0.05$). In addition to APC mutations, all three patients with FGFR1 amplifications achieved a PR with regorafenib. In terms of PFS, mutations in SMAD4 and BRAF suggested a poor response to regorafenib. In addition, patients with a TGF-beta pathway demonstrated a worse PFS while using regorafenib than those without a TGF-beta pathway. This finding could be helpful in determining which patients would have a beneficial response to regorafenib.

In analysis of treatment efficacy, patients with APC mutation were more responsive to regorafenib than those who lacked mutation in the APC gene. Mutations in the APC gene, a negative regulator of Wnt/b-catenin signaling, frequently occur in

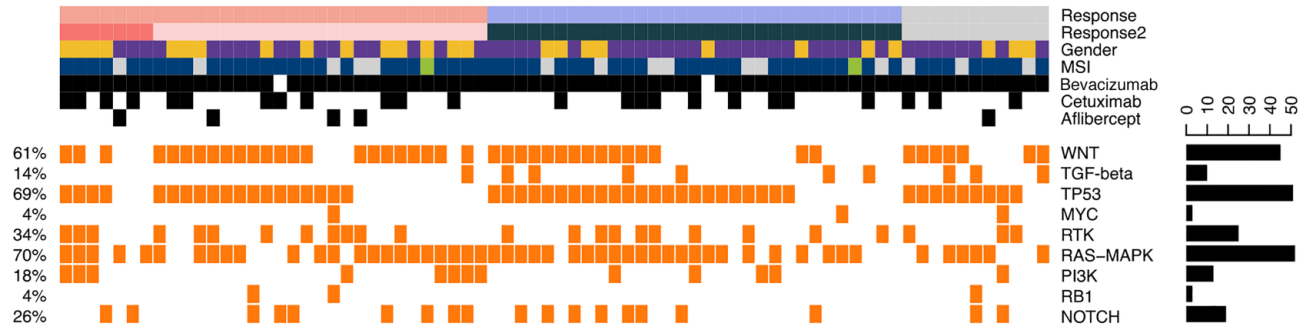

Figure 2. Distribution of signaling pathways involved in patients. If a mutation in the genome was observed, it was considered to affect the pathway.

Table 2. Efficacy of regorafenib.

Tumor response	***n***	**%**
Complete response	0	
Partial response	7	9.2
Stable disease	26	34.2
Progressive disease	32	42.1
NE	11	14.5
Response rate	7	9.2
Disease control rate	33	43.4

NE, Not Evaluable.

CRC.[18,19] Genetically, APC mutation-derived activation of Wnt/b-catenin signaling is required not only for the promotion of CRC, but also for tumor maintenance.[20,21] This finding suggests that APC is a rational therapeutic target in CRC patients. Although regorafenib has potent action as a multi-targeting kinase inhibitor,[7] its effect on signaling of APC is unknown. FGFR1 is a gene that encodes a member of the FGFR family, which includes four receptor tyrosine kinases, FGFR1–4.[22] FGFR1 amplifications were reported in 2.8% of 212 sequenced CRC cases in a TCGA dataset.[23,24] Data regarding FGFR1 amplifications as a novel target in CRC are limited. Regorafenib has been known to target several markers of CRC development through broad kinase inhibition, including FGFR1. Herein, we found that FGFR1 amplification was detected in only three of 74 patients (4.1%), and all of these patients achieved a PR to regorafenib. However, the definition of FGFR1 amplification to regorafenib has been inconsistent among studies. Korphaisarn *et al.* reported that FGFR1 amplification was related to acquired resistance to regorafenib.[12] To obtain reliable data regarding predictive markers of response to regorafenib, further prospective validation of various molecular signals, including APC and FGFR1, must be conducted.

Regorafenib was originally developed as a RAF inhibitor, similar to sorafenib.[25] However, in preclinical and clinical studies, regorafenib demonstrated anti-tumor activity irrespective of RAS and BRAF mutation status.[26–28] Recently, in a subgroup analysis of an LCCC1029 trial, addition of regorafenib to chemotherapy improved survival times among the patient population with KRAS and BRAF dual wild-type CRC. However, patients with BRAF mutation alone did not realize any survival benefit after adding regorafenib. In the present study, patients with BRAF mutation had a significantly worse PFS compared to those without BRAF mutation. Although the present study included only four patients with BRAF mutation, all four demonstrated a tumor response of PD and a short PFS. Based on findings from a LCCC1029 trial and our analysis, the presence of a BRAF mutation might be a negative biomarker for survival in patients treated with regorafenib.

Epithelial to mesenchymal transition (EMT) is associated with tumor invasion and metastasis.[29–32] EMT is also an important resistance factor to anti-cancer therapies and is induced by TGF-beta receptor activation through SMAD4. Previously, one study reported that a patient with SMAD4 mutation showed a long PFS response to regorafenib.[33] Another study showed that a patient with upregulation of the EMT pathway had a better PFS benefit with regorafenib. In the present study, patients with SMAD4 mutation and/or activation of the

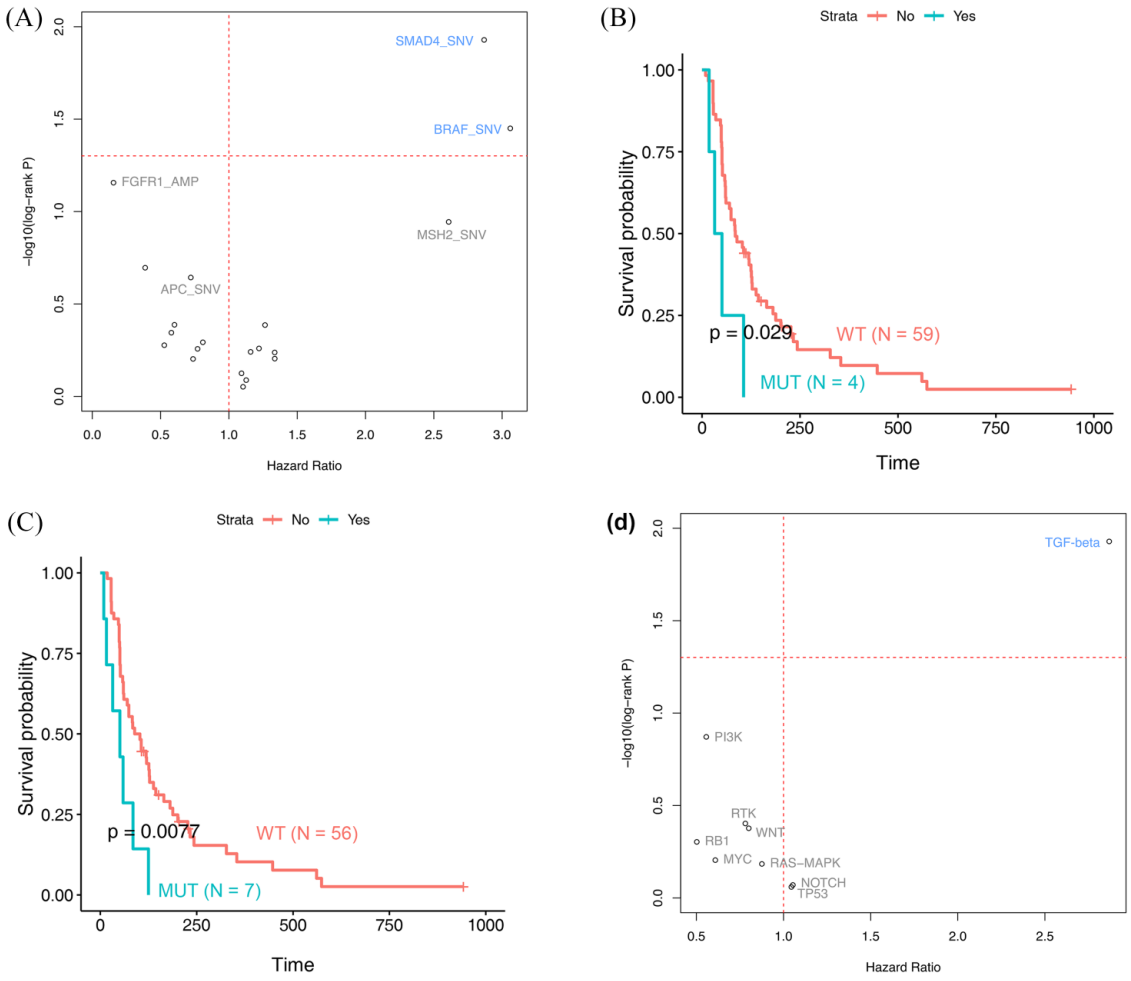

Figure 3. Analysis of the prognostic biomarkers that influence PFS with regorafenib. (A) Cox proportional hazards regression model. BRAF and SMAD4 were significant prognostic factors for a worse PFS to regorafenib. (B) Clinical outcome of regorafebnib treatment according to the presence or absence of BRAF mutations in patients with mCRC. (C) Clinical outcome of regorafebnib treatment according to the presence or absence of SMAD4 mutations in patients with mCRC. (D) Analysis of the prognostic pathway that influences PFS with regorafenib. The TGF-beta pathway showed a significantly poor PFS to regorafenib compared to those without the TGF-beta pathway.
mCRC, metastatic colorectal cancer; PFS, progression-free survival; TGF, Tumor Growth Factor.

TGF-beta pathway showed a worse PFS with regorafenib. This finding is inconsistent with previous studies and may be caused by different co-existing genetic aberrations, varying patient characteristics, and heterogeneity of tumor cells.

In the present study, the response rate to regorafenib was 9.2%. The existing data on single agent treatment with regorafenib with regard to efficacy were heterogeneous. The recent report showed the range of 0.0–6.4% as response rate to regorafenib.[34] The efficacy data of our study seem to be relatively higher compared with previous studies. The heterogeneity of response rate to regorafenib within studies might be caused by different molecular and clinicopathological features of the patient population analyzed in each study. Although we intended to evaluate novel biomarkers, including clinicopathological and molecular values, to predict the outcomes of regorafenib in CRC patients, this study had some limitations. The present study was retrospective in nature. The sample size was small, and there was a lack of consistency in patient characteristics. The NGS, deep targeted sequencing panels, were not sufficient to analyze the molecular characteristics of tumors. In addition, biomarkers found on this study lacked an independent validation cohort. Statistically, we did not conduct the *p*-value

adjustment on multiple testing because, on Cox survival analysis, we selected genetic aberrations that at least three patients had. Thus, the findings in this study must be interpreted with caution.

Conclusion

We defined the molecular characterization of 76 patients treated with regorafenib and identified specific genetic aberrations, such as APC mutation, FGFR1 amplification, SMAD4 mutation, and the TGF-beta pathway, that might be correlated with the anti-tumor activity of regorafenib. The data presented are interesting and deserve further investigation.

Author contributions

Conceptualization, MSL, HJC, YBC and STK; Data curation, HJC; Formal analysis, HJC; Investigation, MSL and HJC; Methodology, MSL; Resources, HJC; Software, HJC; Supervision, YBC and STK; Writing – original draft, MSL, HJC; Writing – review & editing, MSL, HJC, JYH, JL, SHP, JOP, YSP, HYL, WKK, YBC and STK. All authors have read and agreed to the published version of the manuscript.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Supplemental material

Supplemental material for this article is available online.

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