Am J Cancer Res 2024;14(5):2187-2201
www.ajcr.us /ISSN:2156-6976/ajcr0155958

Original Article

The salvage lenvatinib-based regimen provides survival benefit in patients with refractory metastatic colorectal cancer

Yun-Chieh Kao[1,2,4], Mu-Ying Yang[2,4], Wei-Shone Chen[2,3,5], Jeng-Kai Jiang[2,3], Huann-Sheng Wang[2,3], Shih-Ching Chang[2,3], Yuan-Tzu Lan[2,3], Chun-Chi Lin[2,3], Hung-Hsin Lin[2,3], Sheng-Chieh Huang[2,3], Hou-Hsuan Cheng[2,3], Yu-Zu Lin[2,3], Yi-Wen Yang[2,3], Hao-Wei Teng[1,2]

[1]Division of Medical Oncology, Department of Oncology, Taipei Veterans General Hospital, Taipei, Taiwan; [2]School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan; [3]Division of Colon and Rectum Surgery, Department of Surgery, Taipei Veterans General Hospital, Taipei, Taiwan; [4]Division of Medical Education, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan; [5]En Chu Kong Hospital, Xinbei, Taiwan

Received February 18, 2024; Accepted April 27, 2024; Epub May 15, 2024; Published May 30, 2024

Abstract: Salvage treatment for refractory metastatic colorectal cancer (mCRC) has yet to be identified. We aimed to evaluate the efficacy of a salvage lenvatinib-based regimen for refractory mCRC. In total, 371 patients were categorized into lenvatinib-based and non-lenvatinib-based groups. In the lenvatinib-based group, patients who received lenvatinib at a dosage of 10 mg/day were categorized into lenvatinib/chemotherapy and lenvatinib/immunotherapy subgroups. We reported overall survival (OS) and progression-free survival (PFS) using the Kaplan-Meier method. OS1 was used to measure the time from disease progression after TAS-102 and regorafenib treatment to death, while OS2 was used to measure the time from TAS-102 or regorafenib treatment to death. Propensity score matching analysis was employed to compare the characteristics between the lenvatinib-based and non-lenvatinib-based groups. Next-generation sequencing (NGS) information was analyzed using R software. The lenvatinib-based group exhibited longer OS than did the non-lenvatinib-based group (OS1, 11.4 vs. 3.7 months; OS2, 27.2 vs. 8.2 months). The disease control rate (DCR) and objective response rate (ORR) of the lenvatinib-based regimens were 69.4% and 6.1%, respectively. Lenvatinib/chemotherapy and lenvatinib/immunotherapy had similar PFS, OS, DCR, and ORR. The adverse effects were manageable. After propensity score matching, the lenvatinib-based group continued to exhibit significantly longer OS1 and OS2 than did the non-lenvatinib-based group. NGS analysis revealed that *GNAS* and *KRAS* alterations were associated with a worse treatment response and prolonged survival, respectively. In conclusion, a moderate-dose salvage lenvatinib-based regimen demonstrated promising clinical activity and tolerability in treating refractory mCRC.

Keywords: Colorectal cancer (CRC), refractory, lenvatinib, survival, late-line, next-generation sequencing (NGS)

Introduction

CRC ranks as the third most prevalent cancer globally and is the second leading cause of cancer-related fatalities. Although survival rates are increasing, mCRC continues to pose a considerable threat, with an approximate 5-year survival rate of only 14% [1]. Chemotherapy with 5-fluorouracil (5-FU) and leucovorin remains the backbone of treatment regimens. In addition to first- and second-line treatment regimens, new molecularly targeted pharmacological strategies, including VEGF, EGFR, BRAF, and immune checkpoint inhibitors, have been proven to improve survival in certain patient groups [2]. For a small subpopulation with MSI-H, HER-2 amplification [3], *BRAF* mutation, *KRAS G12C* mutation [4], RET fusion, or NTRK fusion [5], specific agents have shown clinical efficacy in these molecular subgroups. Nevertheless, the majority of mCRC patients ultimately develop resistance to standard biochemotherapy.

Studies have shown that regorafenib and TAS-102 improve survival after chemotherapy resis-

https://doi.org/10.62347/YKZB8359

tance. Patients treated with regorafenib monotherapy had a median OS of 6.4 months, whereas those treated with TAS-102 monotherapy had a median OS ranging from 6.5 to 7.8 months. In a recent study, TAS-102 plus bevacizumab demonstrated significant survival benefits, with a median OS of 10.8 months, surpassing the outcome of TAS-102 monotherapy [6-8]. After patients develop drug resistance to regorafenib and TAS-102, there is a lack of clear late-line salvage treatment regimens available; clinicians rely on genetic information, clinical literature, and early results from clinical trials to choose available drugs across different cancer types on the market that may be effective in salvage treatment [9]. The prognosis of patients with MSS mCRC is challenging, with a median OS of less than one year using existing therapies. This highlights a critical unmet need for novel therapeutic options for MSS mCRC patients.

Lenvatinib is a multitargeted inhibitor that suppresses vascular endothelial growth factor receptor (VEGFR) 1-3, fibroblast growth factor receptor (FGFR) 1-4, platelet-derived growth factor receptor (PDGFR) α, and the proto-oncogenes RET and KIT [10]. In the phase II LEMON study, lenvatinib monotherapy demonstrated promising antitumor activity, achieving a median PFS of 3.6 months, a median OS of 7.4 months, and a DCR of 70% in patients with refractory mCRC [11]. In the phase II LEAP-005 study, pembrolizumab, a PD-1 inhibitor, in combination with lenvatinib demonstrated a favorable trend toward improving survival in patients with previously treated MSS mCRC [12]. Recently, the phase III randomized control trial LEAP-017 compared lenvatinib plus pembrolizumab to regorafenib or TAS-102 monotherapy and revealed that lenvatinib/pembrolizumab in combination tended to prolong OS (9.8 months vs. 9.3 months) [13].

In our study, we evaluated the clinical efficacy and safety of moderate-dose lenvatinib (10 mg/day) in combination with chemotherapy or immunotherapy for salvage treatment of refractory mCRC. Furthermore, we analyzed next-generation sequencing (NGS) data from patients treated with a lenvatinib-based regimen to investigate the potential predictive or prognostic roles of these gene alterations.

Methods

Study design and participants

This was a retrospective cohort study, and the data were collected between January 2010 and March 2023 at the Taipei Veterans General Hospital, Taiwan. Eligible patients aged > 20 years with a histologically confirmed diagnosis of mCRC were included. Patients needed to have been previously treated and to have had disease progression on or after or, otherwise, be unable to tolerate standard treatment. Standard treatment was defined as receiving fluoropyrimidine, irinotecan, or oxaliplatin with or without an anti-VEGF monoclonal antibody (e.g., bevacizumab) or an anti-EGFR antibody (e.g., cetuximab or panitumumab) for *RAS* wild-type tumors.

Patients were categorized into groups based on the salvage regimens, which included lenvatinib-based regimens (received TAS-102 and regorafenib, followed by lenvatinib-based regimens), non-lenvatinib-based regimens (received TAS-102 or regorafenib, followed by chemotherapy or palliation care), and lenvatinib prior to receiving TAS-102 or regorafenib (received lenvatinib-based regimens, followed by TAS-102 or regorafenib). Patients in the lenvatinib-based group were further categorized into subgroups: lenvatinib/chemotherapy and lenvatinib/immunotherapy. Patients in the non-lenvatinib-based group were categorized into rechallenge chemotherapy and palliative treatment subgroups. The inclusion flowchart and treatment timeline are presented in Supplementary Figure 1A and 1B, respectively.

Basic patient clinicopathological information, including age, gender, primary tumor location, American Joint Committee on Cancer 7th Edition at presentation (AJCC stage), metastasectomy (curative-intended surgical resection of metastasis during any stage), pathology, histological grading, mucinous component, signet cell component, lymphovascular invasion (LVSI) status, perineural invasion (PNI) status, microsatellite status, *RAS* mutation status, and *BRAF* mutation status, was collected. All the materials and protocols in this study complied with the Declaration of Helsinki and Good Clinical Practice Guidelines. The study protocol was approved by the Ethics Committee and Institutional Review Board of the Taipei Veterans General Hospital.

Treatment

A daily dose of lenvatinib (Lenvima®) of 10 mg was prescribed in the lenvatinib-based group combined with either chemotherapy or immunotherapy until disease progression or unacceptable toxicity occurred. The addition of targeted therapies was permitted and was based on the physician's decisions. Regorafenib (Stivarga®) at 160 mg/day on days 1-21 was administered every 28 days, or dose escalation started at a dose of 80 mg/day on days 1-7, then at 120 mg/day on days 8-14, and then at 160 mg/day on days 15-21. Subsequent cycles of 160 mg/day on days 1-21 were administered every 28 days (ReDOS). TAS-102 (Lonsurf®, trifluridine/tipiracil hydrochloride) every 28 days (twice daily on days 1-5 and days 8-12, with no doses on days 6-7 or days 13-28) was prescribed to the non-lenvatinib-based group. The dosage adjustment of regorafenib and TAS-102 was dependent on the physician's judgment, patient compliance, and adverse effects. Chemotherapy rechallenge was defined as the reinitiation of a combination of oxaliplatin, irinotecan and fluorouracil.

Treatment response assessments

Two types of OS were reported (Supplementary Figure 1B). OS1 was defined as the time from disease progression after TAS-102 and regorafenib treatment to death or censoring, while OS2 was defined as the time from initiation of TAS-102 or regorafenib treatment to death or censoring. PFS was defined as the time from the beginning of the lenvatinib-based regimen to disease progression confirmed by radiological imaging (CT, PET/CT, MRI) or discontinuation due to intolerable toxicity. The evaluation of metastatic disease was based on the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. We recorded the best response after the initiation of treatment in the lenvatinib-based group and in the lenvatinib group prior to receiving TAS-102 or regorafenib. The DCR was calculated as the percentage of patients who achieved CR, PR, or SD. The ORR was calculated as the percentage of patients who achieved CR or PR.

Statistical analysis

The correlations among clinicopathological variables and treatment responses were analyzed using the Chi-square test or Fisher's exact test. Survival curves were generated through Kaplan-Meier methods and the log-rank test. Univariate and multivariate analyses were also conducted using the Cox proportional hazards regression model. Variables with a P value < 0.1 in the univariate analysis were included in the multivariate regression model. Cox regression models were used in the subgroup analysis to evaluate the impact of lenvatinib-based treatment on baseline variables. A two-sided P value < 0.05 was regarded as significant. All the statistical analyses were performed using SPSS 25.0 software (IBM Co., Armonk, NY). OncoPrint and heatmaps of gene alterations according to response were generated with the R ComplexHeatmap package [14].

Propensity score matching

Propensity scores were used to control for selection bias and derived using binary logistic regression to generate a propensity score for each patient who did or did not receive lenvatinib-based regimens. A one-to-one nearest-neighbor matching method between patients receiving lenvatinib-based regimens and those receiving non-lenvatinib-based regimens was achieved through a propensity score matching model, employing a match tolerance of 0.2.

NGS

Some patients in the lenvatinib-based group and in the lenvatinib prior to receiving TAS-102 or regorafenib group receiving NGS were enrolled for bioinformatics analysis. All the NGS data in our cohort were analyzed via the Roche® FoundationONE CDx assay, which included 324 hotspot mutations.

TCGA database analysis

The TCGA-COAD dataset from the TCGA database was used for differential expression analysis (DEA) between samples with mutant or wild-type *RAS* via the TCGABiolink package in the R project (fdr.cut = 0.05, logFC.cut = 1.5, version 2024/1/7) [15]. Gene Ontology analysis and network analysis were performed by using ShinyGO 0.77 (http://bioinformatics.sdstate.edu/go77/).

Results

Patients' baseline characteristics

The baseline characteristics of patients treated with salvage lenvatinib-based or non-lenva-

Table 1. Patients' baseline characteristics

		Non-lenvatinib-based (N = 322)		Lenvatinib-based (N = 44)		*P* value
		N	(%)	N	(%)	
Age	≥ 65	134	(41.6)	9	(20.5)	0.007*
	< 65	188	(58.4)	35	(79.5)	
Gender	Male	185	(57.5)	26	(59.1)	0.837
	Female	127	(42.5)	18	(40.9)	
Stage (AJCC 8th)	I	8	(2.5)	1	(2.3)	0.532
	II	22	(6.8)	1	(2.3)	
	III	74	(23.0)	8	(18.2)	
	IV	218	(67.7)	34	(77.3)	
Primary location	Right	66	(20.5)	6	(13.6)	0.270*
	Left	247	(76.7)	38	(86.4)	
	Multi-location	9	(2.8)	0	(0)	
Metastasectomy	Yes	142	(44.1)	32	(72.7)	0.008*
	No	180	(55.9)	12	(27.3)	
Pathology	Adenocarcinoma	305	(94.7)	42	(97.7)	0.401
	Mucinous adeno	17	(5.3)	1	(2.3)	
Grade	Moderate	251	(78)	37	(84.1)	0.587
	Poor	16	(5)	1	(2.3)	
	NA	55	(17.1)	6	(13.6)	
Mucinous component	Yes	24	(7.5)	5	(11.4)	0.563
	No	243	(75.5)	36	(81.8)	
	NA	55	(17.1)	3	(6.8)	
Signet cell component	Yes	11	(3.4)	0	(0)	0.209
	No	256	(79.5)	37	(84.1)	
	NA	55	(17.1)	7	(15.9)	
LVSI	Yes	125	(38.8)	22	(50)	0.335
	No	134	(41.6)	16	(36.4)	
	NA	63	(19.6)	6	(13.6)	
PNI	Yes	78	(24.2)	15	(34.1)	0.315
	No	181	(56.2)	23	(52.3)	
	NA	63	(19.6)	6	(13.6)	
Microsatellite status	MSS	313	(97.2)	41	(93.2)	0.104*
	MSI-H	0	(0)	1	(2.3)	
	NA	9	(2.8)	2	(4.5)	
RAS	Wild-type	163	(50.6)	19	(43.2)	0.696
	Mutation	158	(49.1)	21	(47.7)	
	NA	1	(0.3)	4	(9.1)	
BRAF	Wild-type	288	(89.4)	39	(88.6)	0.011*
	Mutation	33	(10.2)	3	(6.8)	
	NA	1	(0.3)	2	(4.5)	

Percentages may not add up to exactly 100% due to rounding. Abbreviations: AJCC, American Joint Committee on Cancer staging system; LVSI, lymphovascular invasion; PNI, perineural invasion; mCRC, metastatic colorectal cancer; MSS, Microsatellite Stable; MSI-H, Microsatellite Instability-High; NA, not available. *P value < 0.05.

tinib-based agents are presented in **Table 1**. In the lenvatinib-based group, there were fewer patients aged more than 65 years, more patients who underwent metastasectomy, and fewer patients with *BRAF* mutations than in the non-lenvatinib-based group. The other clinical baseline characteristics were generally well balanced and were not significantly different

between the groups. The baseline characteristics of patients treated with lenvatinib prior to receiving TAS-102 or regorafenib are presented in Supplementary Table 1.

Efficacy by treatment strategy

The survival data, including OS1, OS2, and PFS, are presented in **Figure 1**. In the lenvatinib-based group, the OS1 was significantly longer than that in the non-lenvatinib-based group (11.4 months vs. 3.7 months, P < 0.001) (**Figure 1A**). In the non-lenvatinib-based group, the chemotherapy rechallenge subgroup had longer OS1 than did the palliative treatment subgroup (7.0 months vs. 1.7 months, P < 0.001) (**Figure 1B**). In the lenvatinib-based treatment group, the PFS was 6.2 months (**Figure 1C**), and within the lenvatinib-based group, lenvatinib/chemotherapy and lenvatinib/immunotherapy provided comparable PFS (6.6 months (95% CI 3.6-9.6) vs. 1.9 months (95% CI 0.5-5.3), P = 0.745) (**Figure 1D**) and OS1 (11.4 months (95% CI 7.7-15.1) vs. 14.3 months (95% CI 5.5-23.1), P = 0.554) (Supplementary Figure 2). In the lenvatinib-based group, the OS2 was longer than that in the non-lenvatinib-based group (27.2 months vs. 8.2 months, P < 0.001) (**Figure 1E**), and the chemotherapy rechallenge subgroup exhibited significantly longer OS2 than the palliative treatment subgroup (12.5 months vs. 4.6 months, P < 0.001) (**Figure 1F**).

Antitumor response

The treatment outcomes of patients treated with lenvatinib/chemotherapy and lenvatinib/immunotherapy are presented in **Figure 2A** and **2B**, respectively.

In summary, the DCR was 69.4%, and the ORR was 6.1%. The ORR of lenvatinib/chemotherapy was 5.4%, while the ORR of lenvatinib/immunotherapy was 8.3%; the DCR of lenvatinib/chemotherapy was 73%, while the DCR of lenvatinib/immunotherapy was 58.3% (**Figure 2C**). The overall treatment outcomes of salvage lenvatinib-based regimens and lenvatinib prior to treatment with TAS-102 or regorafenib are presented in Supplementary Figure 3. Moreover, some images of representative cases with regressive change after salvage lenvatinib-based regimen were presented in Supplementary Figure 8.

Prognostic factors impacting OS1

To identify the prognostic factors for OS1, we conducted univariate (**Figure 3A**) and multivariate (**Figure 3B**) analyses. According to univariate analyses, the lenvatinib-based regimen and metastasectomy were identified as favorable prognostic factors, whereas mucinous adenocarcinoma, histological grade 3-4, LVSI, and PNI were considered poor prognostic factors. After controlling for other confounding factors, we found that the lenvatinib-based regimen and metastasectomy status were still independent factors associated with a better prognosis, while the LVSI grade remained an independent factor linked to a worse prognosis. The prognostic factors impacting OS2 (Supplementary Figure 4A and 4B) and OS1 in the lenvatinib-based group (Supplementary Figure 5A and 5B) are also presented.

Subgroup analysis for OS1

To identify potential subgroups that could benefit from salvage lenvatinib-based regimens, we conducted a comprehensive subgroup analysis of various clinical and molecular factors with a Cox proportional hazards model (**Figure 4A**). Within these subgroups, age less than 65 years, initial stage IV disease, adenocarcinoma, moderate-grade tumors, the absence of a mucinous component, primary lesion resection, MSS status, *RAS* gene mutation, and wild-type *BRAF* gene status were identified as favorable indicators for lenvatinib-based regimens. The subgroup analysis for OS2 is presented in Supplementary Figure 6.

Safety

The adverse effects associated with salvage lenvatinib-based regimens are summarized in **Figure 4B**. The most frequent adverse effects were anemia (81.6%), proteinuria (65.3%), and hypothyroidism (51%). Regarding grade 3-4 severity, the most common effects were anemia (14.3%), hypertension (14.3%), and leukopenia (6.1%).

Propensity score matching

Propensity analysis with the one-to-one nearest-neighbor matching method was applied to minimize confounding factors, including age, gender, primary tumor location, AJCC stage, metastasectomy, pathology, histological grade,

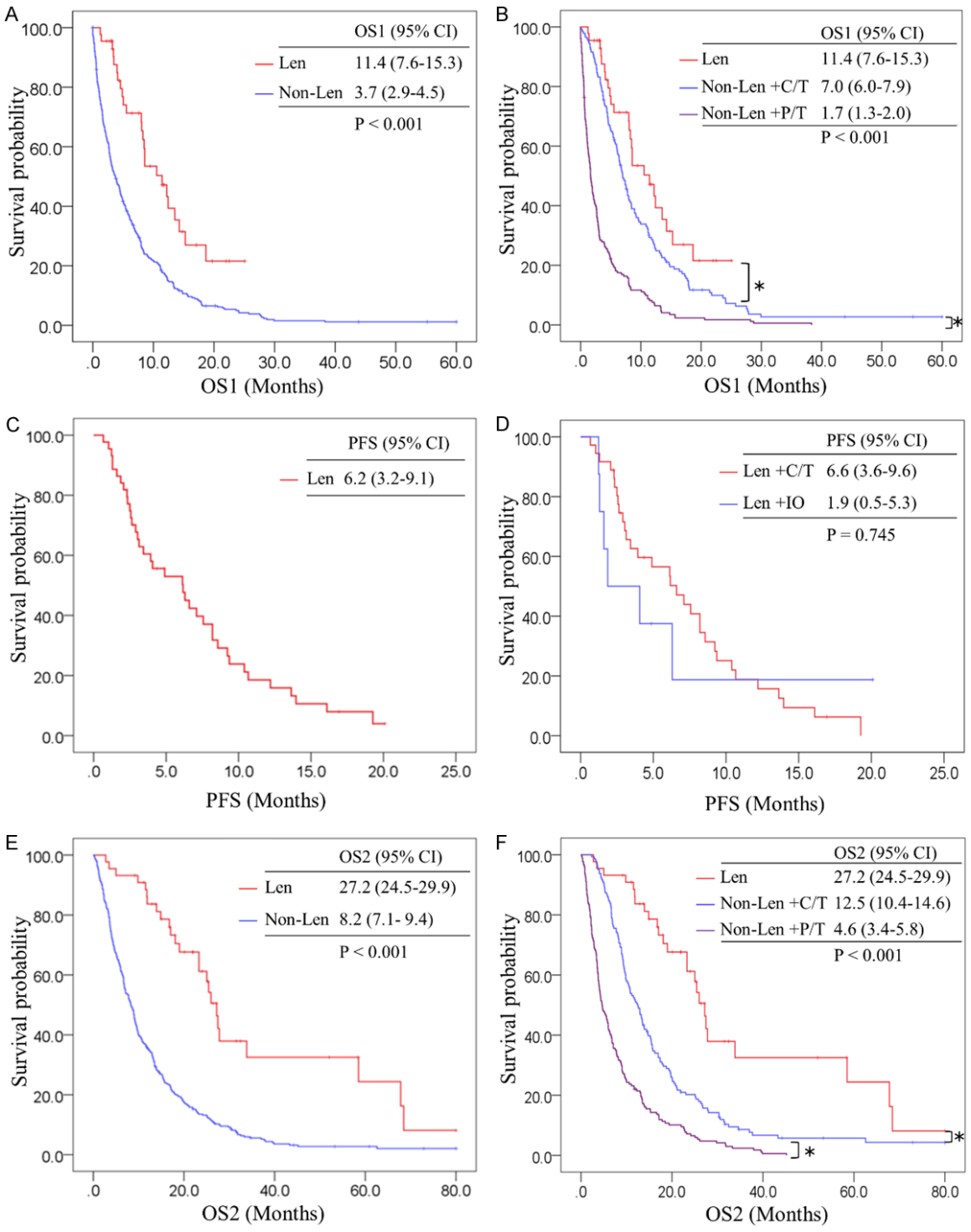
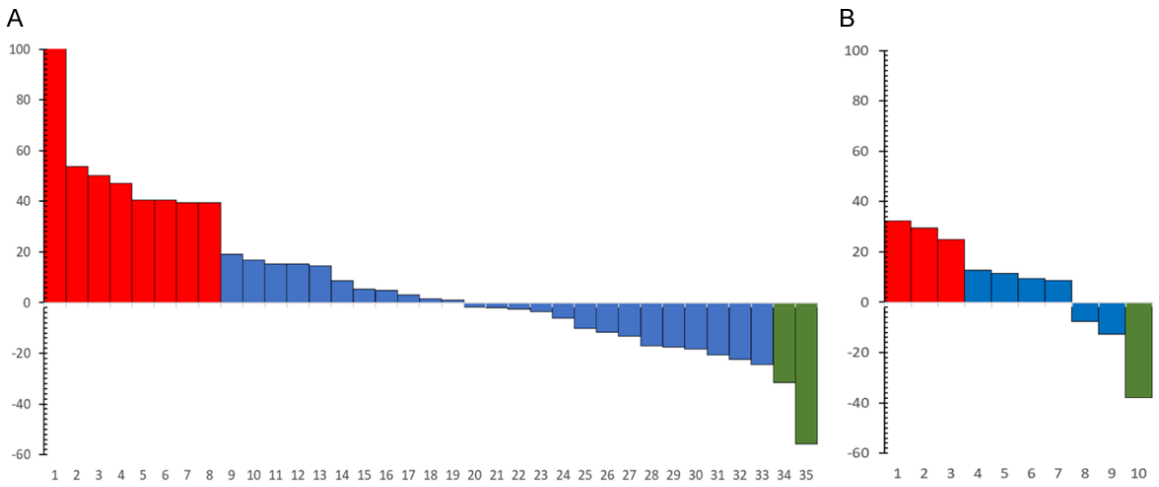


Figure 1. Efficacy by treatment strategy. A. The image represents OS1 by salvage lenvatinib-based regimen or not; B. The image represents the OS1 by different salvage regimens; C. The image represents the PFS of salvage lenvatinib-based regimen; D. The image represents the PFS of salvage lenvatinib-based group by combining C/T or IO; E. The image represents the OS2 by salvage lenvatinib-based regimen or not; F. The image represents the OS2 by different salvage regimens.

mucinous component, signet cell component, LVSI, PNI, microsatellite status, *RAS* mutation status, and *BRAF* mutation status (**Figure 5A**). Finally, 36 patients were matched in each



C

		Len + C/T (N=37)		Len + IO (N=12)		P value	Len Sum (N=49)	
		N	(%)	N	(%)		N	(%)
Best Response	PD	10	(27.0)	5	(41.7)		15	(30.6)
	SD	25	(67.6)	6	(50.0)		31	(63.2)
	PR	2	(5.4)	1	(8.3)		3	(6.1)
DCR		27	(73)	7	(58.3)		34	(69.4)
ORR		2	(5.4)	1	(8.3)		3	(6.1)
PFS (95% CI)		6.6 (3.6-9.6)		1.9 (0.5-5.3)		0.745	6.1 (3.4-8.9)	
OS1 (95% CI)		11.4 (7.7-5.1)		14.3 (5.5-23.1)		0.554	12.2 (7.0-17.4)	

Figure 2. Antitumor response in patients with salvage lenvatinib-based regimen and lenvatinib prior to receiving TAS-102 or regorafenib. A. The image shows the best response of lenvatinib/chemotherapy by RECIST version 1.1. B. The image shows the best response of lenvatinib/immunotherapy by RECIST version 1.1. C. The image shows the treatment outcomes, the distribution of response and survival data.

group, and the abovementioned factors appeared to be well matched between these two groups (**Table 2**). After matching, the lenvatinib-based group continued to exhibit significantly longer OS1 (8.6 vs. 3.8 months) and OS2 (27.2 vs. 13.8 months) than did the non-lenvatinib-based group (**Figure 5B**, **5C**).

NGS

OncoPrint on column clustering by response is shown in **Figure 6A**. A heatmap of column-supervised clustering by response and row-unsupervised clustering by genes having an impact on survival in the KM plots is shown in **Figure 6B**. The Chi-square test revealed that GNAS alterations were associated with a worse treatment response, although this difference was not significant in the survival analysis. Patients with *KRAS* mutations exhibited significantly better survival (P = 0.017). Conversely, patients with mutations in *CDH1, MCL1, PDK1, CHEK1, KLHL6, PIK3CB, KEL, CARD11, CD79A, CSF1R, BRAF, MERTK, FGF4, SNCAIP,* or *RAC1* had significantly worse survival (**Figure 6C**).

TCGA database analysis

The 238 *RAS* wild-type and *BRAF* wild-type samples and 180 *RAS* mutant and *BRAF* wild-type samples were subjected to DEA. Finally, 651 differentially expressed genes were identified, and their distributions are shown in a volcano plot (**Figure 6D**). In addition, the top 30 significant pathways in the Gene Ontology Molecular Function category are shown as a dot plot (**Figure 6E**). The network analysis of these significant pathways is shown in Supplementary Figure 7. This report highlights

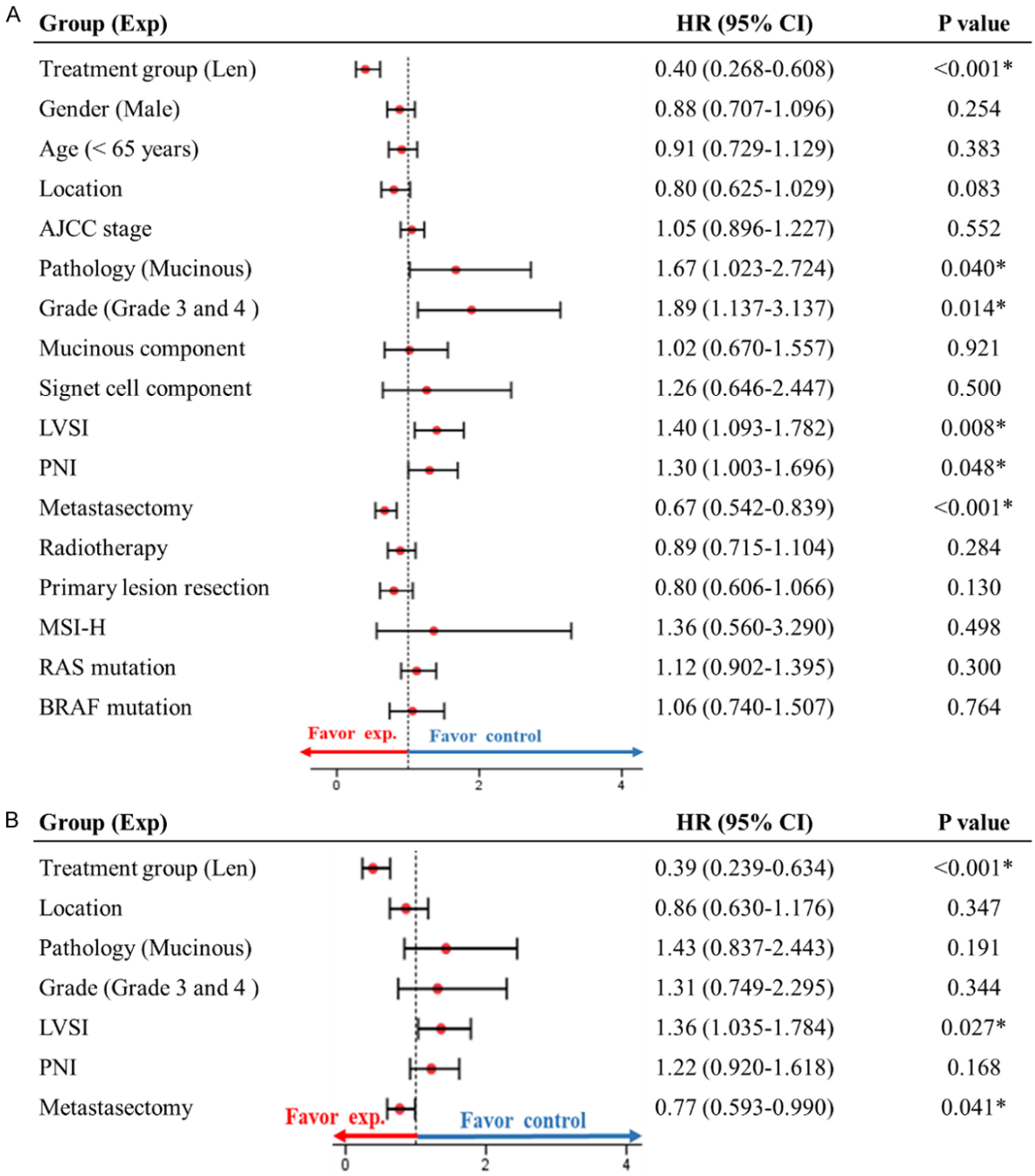


Figure 3. Exploring clinical-pathological factors impacting OS1 in mCRC patients. A. The image represents the univariate analysis of OS1 impacting factors; B. The image represents the multivariate analysis of OS1 impacting factors.

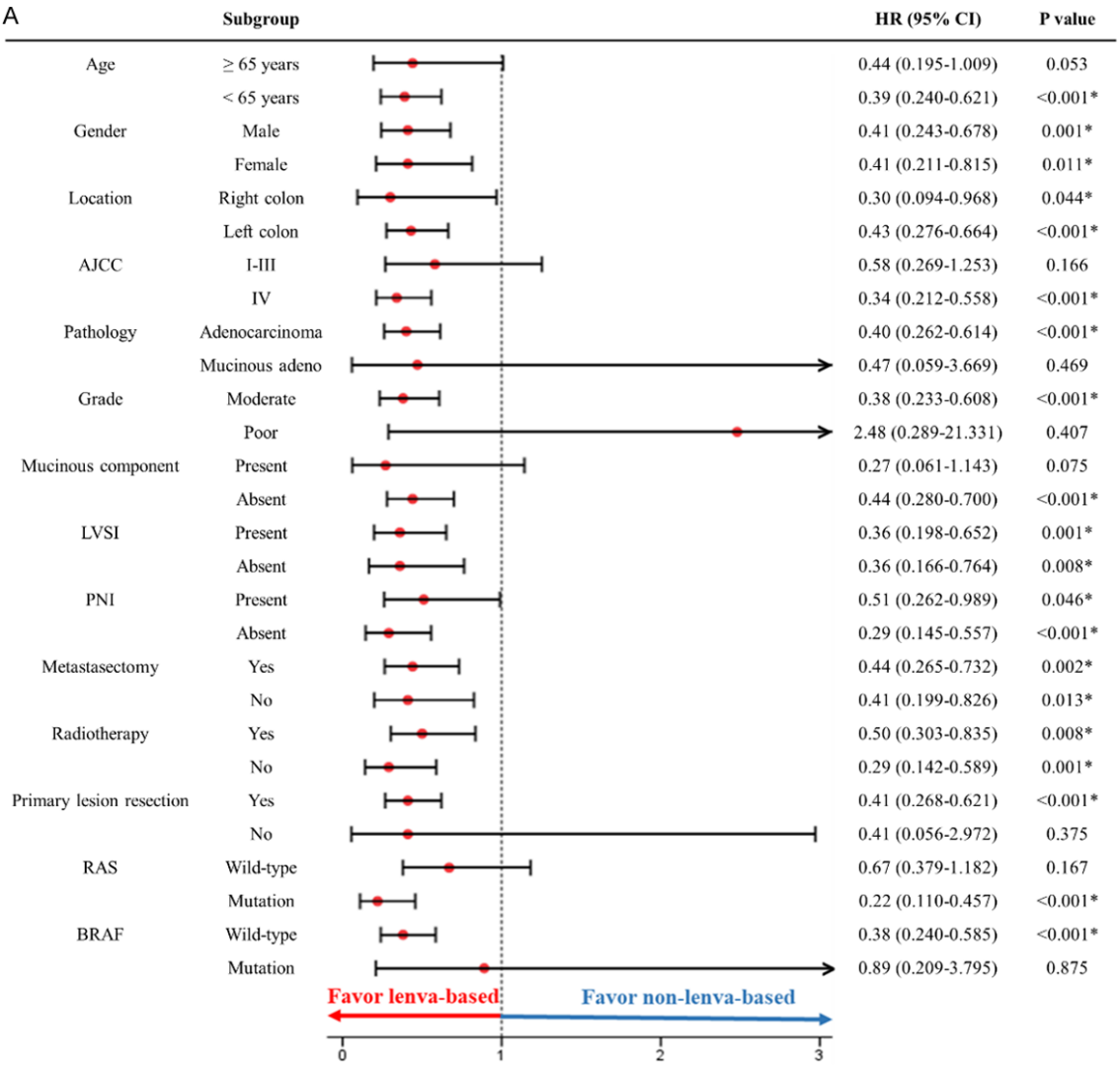
the importance of the fibroblast growth factor (FGF) pathway.

Discussion

Our study successfully demonstrated that a salvage lenvatinib-based regimen conferred a survival benefit in patients with refractory mCRC, particularly when it was administered at a moderate dose of 10 mg/day. To our knowledge, no comparable clinical trial has been conducted to date.

Patients receiving the salvage lenvatinib-based regimen had an OS1 of 11.4 months after becoming refractory to TAS-102 and rego-

A



B

	Any grade		Grade 3-4	
	N	(%)	N	(%)
Leukopenia	19	(38.8)	3	(6.1)
Anemia	40	(81.6)	7	(14.3)
Thrombocytopenia	13	(26.5)	0	(0)
Anorexia	3	(6.1)	0	(0)
Elevated Creatinine	4	(8.2)	0	(0)
Elevated ALT	13	(26.5)	1	(2.0)
Elevated AST	7	(14.3)	0	(0)
Diarrhea	2	(4.1)	0	(0)
Proteinuria	32	(65.3)	4	(8.2)
Hypertension	24	(49.0)	7	(14.3)
Hypothyroidism	25	(51.0)	0	(0)
Elevated Lipase	4	(8.2)	2	(4.1)
Weight loss	16	(32.7)	0	(0)

Figure 4. Subgroup analysis of OS1 in mCRC patients and adverse effects. A. The forest plot represents the magnitude of the hazard ratio for different subgroups. B. The table showed the summary of adverse events in patients treated with salvage lenvatinib-based regimen and lenvatinib prior to receiving TAS-102 or regorafenib. The adverse effects grading was based on common terminology criteria for adverse events (CTCAE) version 5.0.

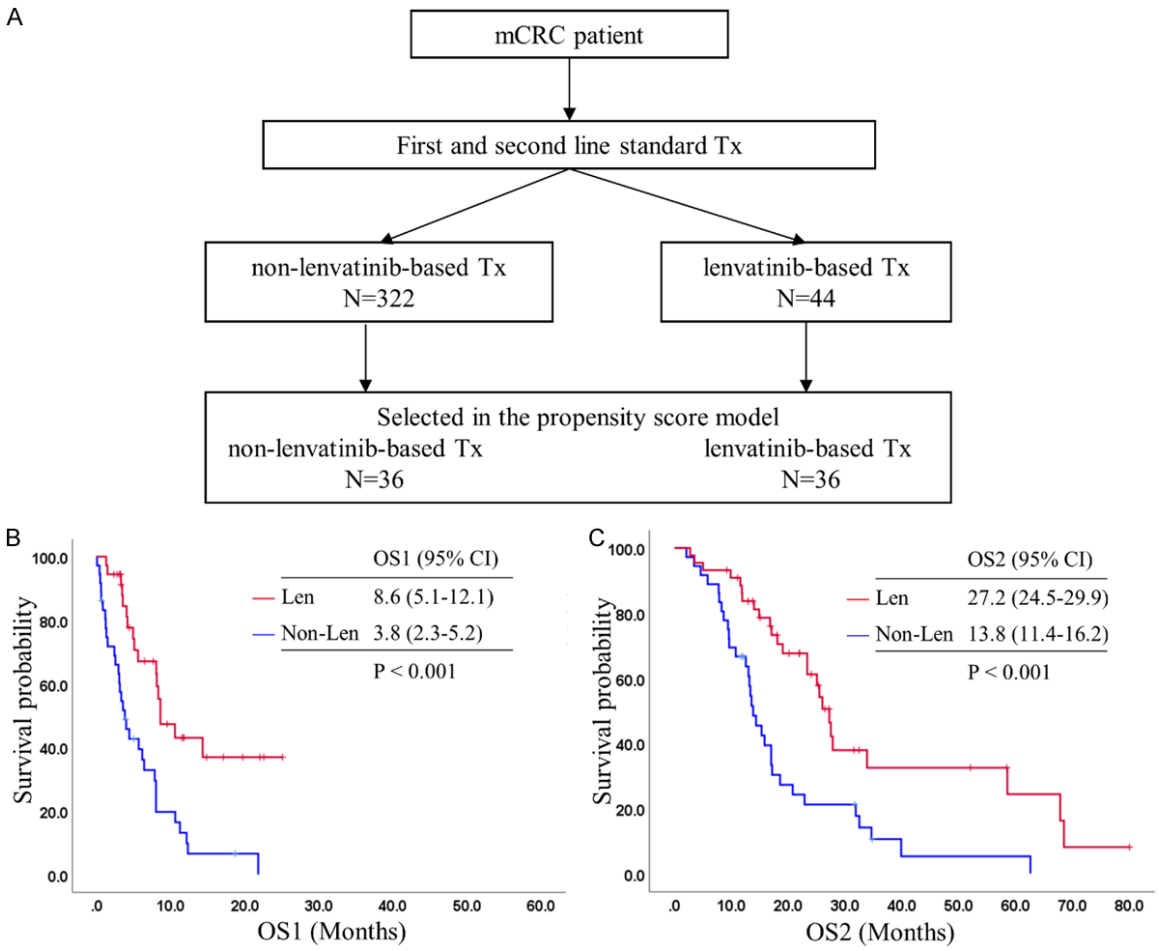


Figure 5. Survival analysis of lenvatinib-based versus non-lenvatinib-based groups after propensity score matching. A. 36 pairs of matched patients were selected for analysis by the propensity score model using the one-to-one nearest-neighbor matching method to minimize the confounding factors. B. The image represents OS1 by lenvatinib-based regimen or not after propensity score matching. C. The image represents OS2 by lenvatinib-based regimen or not after propensity score matching.

rafenib. Our findings were indirectly confirmed by the LEMON study, which suggested that lenvatinib monotherapy provided an OS of 7.4 months and a PFS of 3.6 months in previously treated mCRC patients treated with at least 2 lines of chemotherapy (and/or TAS-102) [11]. Our results suggested that a lenvatinib-based regimen prolongs survival after patients are refractory to regorafenib and TAS-102.

Patients receiving the salvage lenvatinib-based regimen demonstrated an OS2 of 27.2 months after becoming refractory to at least two lines of chemotherapy. The results of previous studies, including the CORRECT, RECOURSE, SUNLIGHT, and LEAP-017 studies, support our findings. The current survival outcomes of patients receiving standard third-line therapy are still unsatisfactory: the OS of patients receiving regorafenib monotherapy was 6.4 months in the CORRECT trial, the OS of patients receiving TAS-102 monotherapy was 6.5-7.8 months in the RECOURSE trial, and the OS of patients receiving TAS-102 plus bevacizumab was 10.8 months in the SUNLIGHT trial. In the LEAP-017 trial, lenvatinib plus pembrolizumab was associated with longer survival than regorafenib or TAS-102 (9.8 months vs. 9.3 months) [13].

Although heterogeneity in baseline characteristics was observed between the lenvatinib-based group and the non-lenvatinib-based group, the above results encouraged us to assess the actual efficacy of the lenvatinib-based regimen using a propensity score model.

Table 2. Patients' baseline characteristics after propensity score matching

		Non-lenvatinib-based (N = 36)		Lenvatinib-based (N = 36)		*P* value
		N	(%)	N	(%)	
Age	≥ 65	15	(41.7)	7	(19.4)	0.072
	< 65	21	(58.3)	29	(80.6)	
Gender	Male	20	(55.6)	20	(55.6)	1.000
	Female	16	(44.4)	16	(44.4)	
Stage (AJCC 8th)	I	1	(2.8)	1	(2.8)	0.822
	II	2	(5.6)	1	(2.8)	
	III	7	(19.4)	5	(13.9)	
	IV	26	(72.2)	29	(80.6)	
Primary location	Right	6	(16.7)	4	(11.1)	0.735
	Left	30	(83.3)	32	(88.9)	
Metastasectomy	Yes	17	(47.2)	26	(72.2)	0.054
	No	19	(52.8)	10	(27.8)	
Pathology	Adenocarcinoma	33	(91.7)	35	(97.2)	0.614
	Mucinous adeno	3	(8.3)	1	(2.8)	
Grade	Moderate	34	(94.4)	35	(97.2)	1.000
	Poor	2	(5.6)	1	(2.8)	
Mucinous component	Yes	9	(25)	5	(13.9)	0.372
	No	27	(75)	31	(86.1)	
Signet cell component	Yes	3	(8.3)	0	(0)	0.239
	No	33	(91.7)	36	(100)	
LVSI	Yes	16	(44.4)	20	(55.6)	0.480
	No	20	(55.6)	16	(44.4)	
PNI	Yes	10	(27.8)	15	(41.7)	0.322
	No	26	(72.2)	21	(58.3)	
Microsatellite status	MSS	36	(100)	36	(100)	-#
	MSI-H	0	(0)	0	(0)	
RAS	Wild-type	15	(41.7)	16	(44.4)	1.000
	Mutation	21	(58.3)	20	(55.6)	
BRAF	Wild-type	36	(100)	33	(91.7)	0.239
	Mutation	0	(0)	3	(8.3)	

Percentages may not add up to exactly 100% due to rounding. Abbreviations: AJCC, American Joint Committee on Cancer staging system; LVSI, lymphovascular invasion; PNI, perineural invasion; mCRC, metastatic colorectal cancer; MSS, Microsatellite Stable; MSI-H, Microsatellite Instability-High; NA, not available. #*P* value was unable to calculate due to zero occurrence.

After propensity score matching, the lenvatinib-based group consistently demonstrated significantly longer OS1 and OS2 than did the non-lenvatinib-based group.

Notably, lenvatinib/chemotherapy and lenvatinib/immunotherapy demonstrated similar efficacy in terms of PFS and OS1 (**Figure 1D**, Supplementary Figure 2). The efficacy of the lenvatinib/chemotherapy regimen may have provided a greater survival benefit than the lenvatinib/immunotherapy regimen. Several preclinical animal studies have suggested that lenvatinib plus chemotherapy has synergistic antitumor effects. Hepatocellular carcinoma (HCC) mouse model studies demonstrated that lenvatinib has immunomodulatory effects on T-cell inflammation, and lenvatinib combined with chemotherapy has synergistic antitumor effects on the phosphorylation of VEGFR, RET, and ERK [16, 17]. Previous research has demonstrated that immune checkpoint inhibitor therapy alone has limited efficacy in patients with MSS CRC [18]. The long tails and early crossover features observed in the OS curve of the lenvatinib/pembrolizumab group were consis-

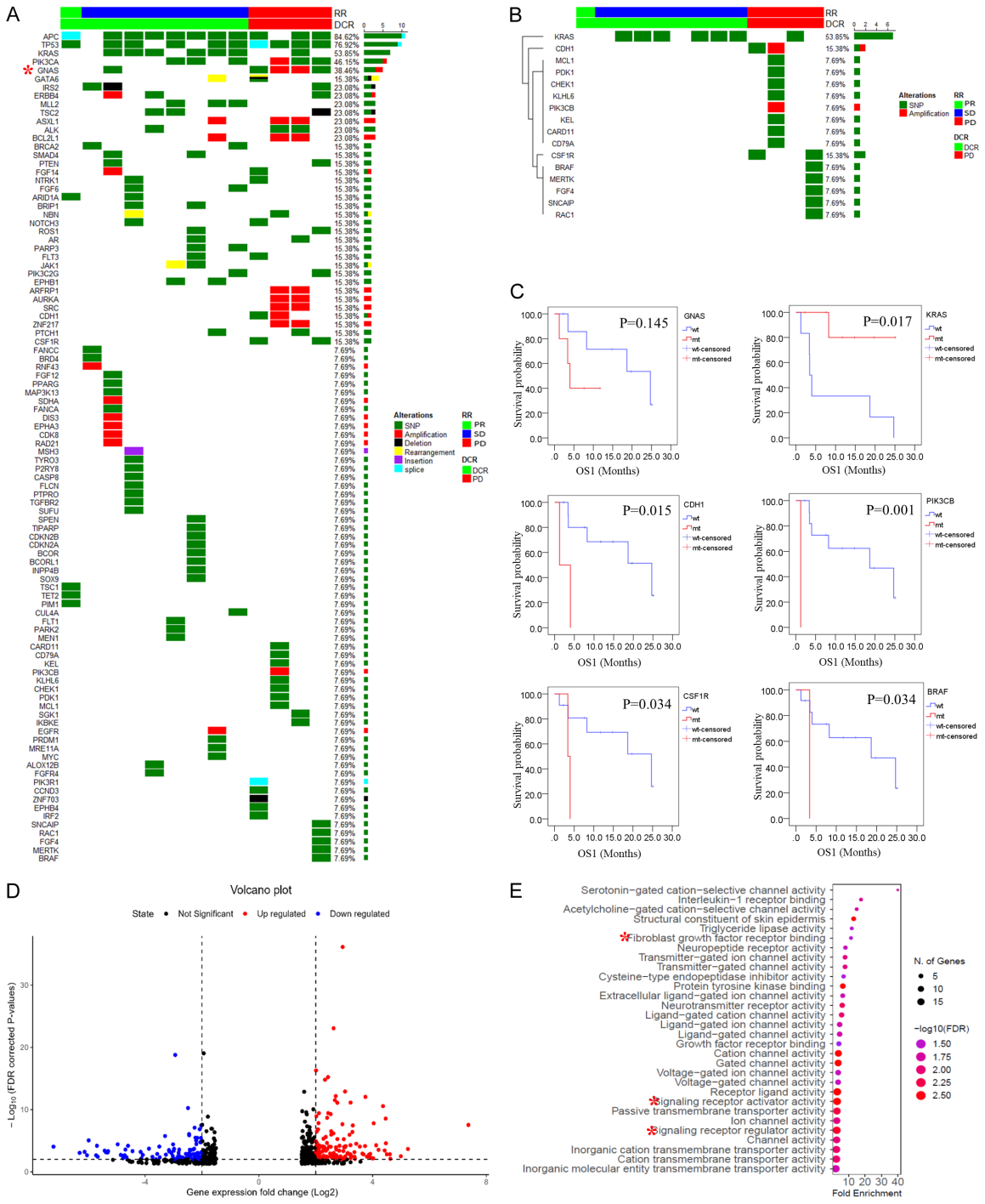


Figure 6. NGS analysis in patients treated with salvage lenvatinib-based regimen and lenvatinib prior to TAS-102 or regorafenib. A. The image represents the OncoPrint on column clustering by response; B. The image represents the heatmap on column supervised clustering by response and row unsupervised clustering by genes having impact on survival; C. The image represents the survival plots of the gene alteration hotspots with significance; D. The image represents the volcano plot of 651 different expression genes and the distribution; E. The image represents the dot plot of the top 30 significant pathway in Gene Ontology Molecular Function.

tent with previous studies of immune checkpoint inhibitors [19].

To avoid the confounding bias, patients in the non-lenvatinib following chemotherapy rechal-

lenge subgroup were isolated and compared with those in the salvage lenvatinib-based group (**Figure 1B**, **1F**). The results showed that patients in the lenvatinib-based group still had the longest survival, and patients in the chemotherapy rechallenge subgroup had longer survival than did patients in the palliative treatment subgroup. Previous studies have suggested that chemotherapy rechallenge has better treatment outcomes than supportive care [20] and is sometimes even more effective than regorafenib in the third-line treatment of mCRC [21].

The survival benefit of lenvatinib-based regimens mainly stems from their ability to control disease. In summary, the DCR was 69.4%, and the ORR was 6.1%. Lenvatinib/chemotherapy achieved a DCR of 73%, while lenvatinib/immunotherapy had a lower DCR of 58.3%; the ORR of lenvatinib/immunotherapy was 8.3%, whereas lenvatinib/chemotherapy yielded an ORR of 5.4%. In the LEMON study, the DCR was 70.0%, and the ORR was 6.7% with lenvatinib monotherapy [11]. In the LEAP-005 trial, the DCR was 47%, and the ORR was 22% in the MSS mCRC cohort [12]. In the LEAP-017 study, the lenvatinib/pembrolizumab combination achieved a DCR of 63.1%, while the ORR was 10.4% [13]. The DCR of lenvatinib/chemotherapy observed in our study surpassed the results reported in these trials, emphasizing the notable efficacy of lenvatinib in combination with chemotherapy in achieving disease control. A comparison of these studies is summarized in Supplementary Table 2.

The most frequent adverse effects observed in our study were anemia (81.6%), proteinuria (65.3%), and hypothyroidism (51%). Regarding grade 3-4 severity, the most common effects were anemia (14.3%), hypertension (14.3%), and leukopenia (6.1%). Our results revealed a lower incidence of hypertension and proteinuria but a greater incidence of anemia and hypothyroidism in our study than in the LEMON study. This could be partially attributed to the relatively lower dose of lenvatinib (10 mg/day). Nevertheless, it was difficult to distinguish between the side effects caused by lenvatinib itself and those induced by concurrent chemotherapy or immunotherapy.

According to the NGS analysis (**Figure 6**), *GNAS* mutation was the only gene alteration correlated with a worse treatment response. A meta-analysis suggested that *GNAS* mutations are significantly linked with poor prognosis and treatment failure in CRC patients [22]. Survival analyses revealed that patients with *KRAS* mutations had significantly longer OS1 ($P = 0.017$), suggesting that the *RAS* mutation is a predictive marker for better treatment outcomes in patients receiving lenvatinib-based regimens. These findings align with the results of our subgroup analysis, which revealed that patients in the *RAS* mutation subgroup tended to benefit from the salvage lenvatinib-based regimen (**Figure 4A**). A human CRC cell line study suggested that lenvatinib significantly delays the growth of *KRAS*-mutated CRC xenografts and decreases the density of tumor-associated vessels in the microenvironment but not during tumor regression [23]. This observation provides insight into why the combination of lenvatinib with chemotherapy or immunotherapy has superior efficacy to that of lenvatinib monotherapy. In the TCGA database analysis, we found that *RAS* mutations were associated with FGF signaling pathways. The FGF pathway is one of many major pathways targeted by lenvatinib. However, the underlying relationship between *RAS* mutations and lenvatinib needs to be better understood to improve patient selection in subsequent studies.

There were several limitations to our study. First, the retrospective design of the study may have led to inevitable selection bias. Patients who were able to receive late-line therapies had better general conditions and were more willing to receive treatment. Second, the data were collected from a single hospital, and a larger multicenter, randomized study is needed to validate our findings. Third, the limited use of NGS data may lead to extreme values and statistical bias. Future research may explore these aspects to provide a more comprehensive understanding of the treatment landscape.

Conclusion

The moderate-dose salvage lenvatinib-based regimen showed promising clinical activity and manageable adverse events in patients with refractory mCRC as a last-line salvage therapy. The combination of these two regimens with chemotherapy or immunotherapy had comparable efficacy. Moreover, NGS gene information offered insights into predictive and prognostic

gene alterations, aiding in the precise selection of patients for salvage lenvatinib-based regimens.

Acknowledgements

This study would like to thank Miss. Chian-You Wu from Institute of Food Safety and Health Risk Assessment, National Yang Ming Chiao Tung University, Taipei, Taiwan and Dr. Hsiang-Tsui Wang from Institute of Pharmacology, College of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan for their contributions.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hao-Wei Teng, Division of Medical Oncology, Department of Oncology, Taipei Veterans General Hospital, No. 201, Section 2, Shipai Road, Taipei 11217, Taiwan. Tel: +886-953255179; E-mail: hwteng1971@gmail.com

References

[1] Rumpold H, Niedersüß-Beke D, Heiler C, Falch D, Wundsam HV, Metz-Gercek S, Piringer G and Thaler J. Prediction of mortality in metastatic colorectal cancer in a real-life population: a multicenter explorative analysis. BMC Cancer 2020; 20: 1149.

[2] Glimelius B, Stintzing S, Marshall J, Yoshino T and de Gramont A. Metastatic colorectal cancer: advances in the folate-fluoropyrimidine chemotherapy backbone. Cancer Treat Rev 2021; 98: 102218.

[3] Ahcene Djaballah S, Daniel F, Milani A, Ricagno G and Lonardi S. HER2 in colorectal cancer: the long and winding road from negative predictive factor to positive actionable target. Am Soc Clin Oncol Educ Book 2022; 42: 1-14.

[4] Yaeger R, Weiss J, Pelster MS, Spira AI, Barve M, Ou SI, Leal TA, Bekaii-Saab TS, Paweletz CP, Heavey GA, Christensen JG, Velastegui K, Kheoh T, Der-Torossian H and Klempner SJ. Adagrasib with or without cetuximab in colorectal cancer with mutated KRAS G12C. N Engl J Med 2023; 388: 44-54.

[5] Pietrantonio F, Di Nicolantonio F, Schrock AB, Lee J, Morano F, Fucà G, Nikolinakos P, Drilon A, Hechtman JF, Christiansen J, Gowen K, Frampton GM, Gasparini P, Rossini D, Gigliotti C, Kim ST, Prisciandaro M, Hodgson J, Zaniboni A, Chiu VK, Milione M, Patel R, Miller V, Bardelli A, Novara L, Wang L, Pupa SM, Sozzi G, Ross J, Di Bartolomeo M, Bertotti A, Ali S, Trusolino L, Falcone A, de Braud F and Cremolini C. RET fusions in a small subset of advanced colorectal cancers at risk of being neglected. Ann Oncol 2018; 29: 1394-1401.

[6] Grothey A, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, Humblet Y, Bouché O, Mineur L, Barone C, Adenis A, Tabernero J, Yoshino T, Lenz HJ, Goldberg RM, Sargent DJ, Cihon F, Cupit L, Wagner A and Laurent D; CORRECT Study Group. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet 2013; 381: 303-312.

[7] Mayer RJ, Van Cutsem E, Falcone A, Yoshino T, Garcia-Carbonero R, Mizunuma N, Yamazaki K, Shimada Y, Tabernero J, Komatsu Y, Sobrero A, Boucher E, Peeters M, Tran B, Lenz HJ, Zaniboni A, Hochster H, Cleary JM, Prenen H, Benedetti F, Mizuguchi H, Makris L, Ito M and Ohtsu A; RECOURSE Study Group. Randomized trial of TAS-102 for refractory metastatic colorectal cancer. N Engl J Med 2015; 372: 1909-1919.

[8] Prager GW, Taieb J, Fakih M, Ciardiello F, Van Cutsem E, Elez E, Cruz FM, Wyrwicz L, Stroyakovskiy D, Pápai Z, Poureau PG, Liposits G, Cremolini C, Bondarenko I, Modest DP, Benhadji KA, Amellal N, Leger C, Vidot L and Tabernero J; SUNLIGHT Investigators. Trifluridine-tipiracil and bevacizumab in refractory metastatic colorectal cancer. N Engl J Med 2023; 388: 1657-1667.

[9] Shin AE, Giancotti FG and Rustgi AK. Metastatic colorectal cancer: mechanisms and emerging therapeutics. Trends Pharmacol Sci 2023; 44: 222-236.

[10] Tohyama O, Matsui J, Kodama K, Hata-Sugi N, Kimura T, Okamoto K, Minoshima Y, Iwata M and Funahashi Y. Antitumor activity of lenvatinib (e7080): an angiogenesis inhibitor that targets multiple receptor tyrosine kinases in preclinical human thyroid cancer models. J Thyroid Res 2014; 2014: 638747.

[11] Iwasa S, Okita N, Kuchiba A, Ogawa G, Kawasaki M, Nakamura K, Shoji H, Honma Y, Takashima A, Kato K, Hamaguchi T, Boku N and Yamada Y. Phase II study of lenvatinib for metastatic colorectal cancer refractory to standard chemotherapy: the LEMON study (NCCH1503). ESMO Open 2020; 5: e000776.

[12] Perez-Fidalgo JA and Martinelli E. Lenvatinib plus pembrolizumab a new effective combination of targeted agents. ESMO Open 2023; 8: 101157.

[13] Kawazoe A, Xu R, Passhak M, Teng H, Shergill A, Gumus M, Qvortrup C, Stintzing S, Towns K, Kim T, Shiu K, Cundom J, Ananda S, Lebedinets A, Fu R, Jain R, Adelberg D, Heinemann V,

Yoshino T and Élez E. LBA-5 Lenvatinib plus pembrolizumab versus standard of care for previously treated metastatic colorectal cancer (mCRC): the phase 3 LEAP-017 study. Ann Oncol 2023; 34: S179.

[14] Gu Z, Eils R and Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics 2016; 32: 2847-2849.

[15] Colaprico A, Silva TC, Olsen C, Garofano L, Cava C, Garolini D, Sabedot TS, Malta TM, Pagnotta SM, Castiglioni I, Ceccarelli M, Bontempi G and Noushmehr H. TCGAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. Nucleic Acids Res 2016; 44: e71.

[16] Kimura T, Kato Y, Ozawa Y, Kodama K, Ito J, Ichikawa K, Yamada K, Hori Y, Tabata K, Takase K, Matsui J, Funahashi Y and Nomoto K. Immunomodulatory activity of lenvatinib contributes to antitumor activity in the Hepa1-6 hepatocellular carcinoma model. Cancer Sci 2018; 109: 3993-4002.

[17] Wang M, Yao X, Bo Z, Zheng J, Yu H, Xie X, Lin Z, Wang Y, Chen G and Wu L. Synergistic effect of lenvatinib and chemotherapy in hepatocellular carcinoma using preclinical models. J Hepatocell Carcinoma 2023; 10: 483-495.

[18] Sahin IH, Ciombor KK, Diaz LA, Yu J and Kim R. Immunotherapy for microsatellite stable colorectal cancers: challenges and novel therapeutic avenues. Am Soc Clin Oncol Educ Book 2022; 42: 1-12.

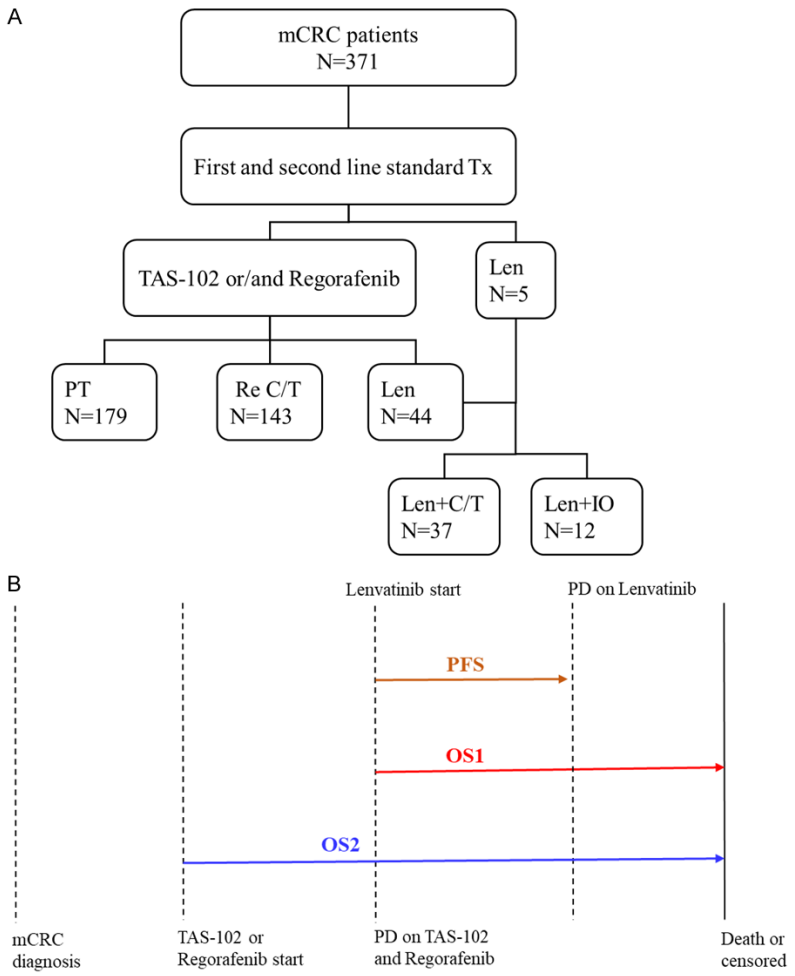
[19] Filleron T, Bachelier M, Mazieres J, Pérol M, Meyer N, Martin E, Mathevet F, Dauxois JY, Porcher R and Delord JP. Assessment of treatment effects and long-term benefits in immune checkpoint inhibitor trials using the flexible parametric cure model: a systematic review. JAMA Netw Open 2021; 4: e2139573.

[20] Suenaga M, Mizunuma N, Matsusaka S, Shinozaki E, Ozaka M, Ogura M and Yamaguchi T. Phase II study of reintroduction of oxaliplatin for advanced colorectal cancer in patients previously treated with oxaliplatin and irinotecan: RE-OPEN study. Drug Des Devel Ther 2015; 9: 3099-3108.

[21] Köstek O, Hacıoğlu MB, Sakin A, Demir T, Sarı M, Ozkul O, Araz M, Doğan AF, Demircan NC, Uzunoğlu S, Çiçin İ and Erdoğan B. Regorafenib or rechallenge chemotherapy: which is more effective in the third-line treatment of metastatic colorectal cancer? Cancer Chemother Pharmacol 2019; 83: 115-122.

[22] Afolabi HA, Salleh SM, Zakaria Z, Ch'ng ES, Mohd Nafi SN, Abdul Aziz AAB, Irekeola AA, Wada Y and Al-Mhanna SB. A GNAS gene mutation's independent expression in the growth of colorectal cancer: a systematic review and meta-analysis. Cancers (Basel) 2022; 14: 5480.

[23] Wiegering A, Korb D, Thalheimer A, Kämmerer U, Allmanritter J, Matthes N, Linnebacher M, Schlegel N, Klein I, Ergün S, Germer CT and Otto C. E7080 (lenvatinib), a multi-targeted tyrosine kinase inhibitor, demonstrates antitumor activities against colorectal cancer xenografts. Neoplasia 2014; 16: 972-981.

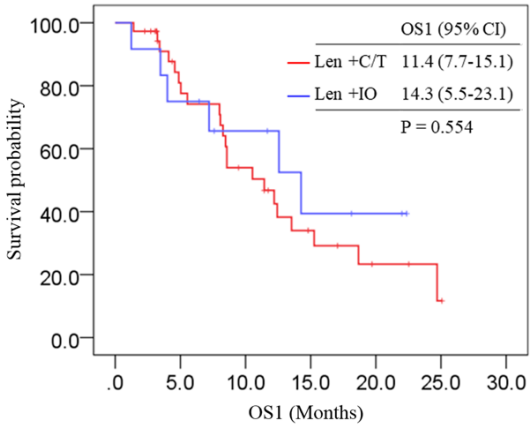


Supplementary Figure 1. Treatment flowchart and timeline of mCRC patients. A. Treatment flowchart of mCRC patients. B. Treatment timeline of mCRC patients. Abbreviations: mCRC, metastatic colorectal cancer; Tx, treatment; Len, lenvatinib; PD, disease progression; Re, re-challenge; C/T, chemotherapy; P/T, palliative therapy; IO, immunotherapy.

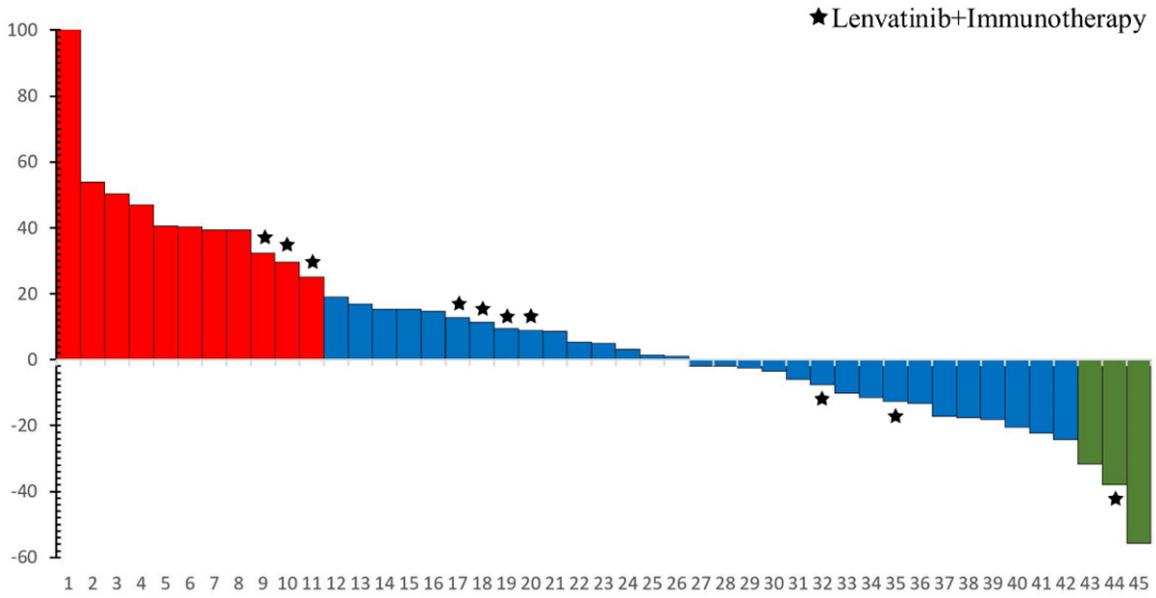
Supplementary Table 1. Baseline characteristics of patients with lenvatinib prior to receiving TAS-102 or regorafenib

		Lenvatinib prior to TAS-102 or regorafenib (N = 5)	
		N	(%)
Age	≥ 65	0	(0)
	< 65	5	(100)
Gender	Male	3	(60)
	Female	2	(40)
Stage (AJCC ^{7th})	I	1	(20)
	II	0	(0)
	III	1	(20)
	IV	3	(60)
Primary tumor location	Right	2	(40)
	Left	3	(60)
	Multi-location	0	(0)
Metastasectomy	Yes	3	(60)
	No	2	(40)
Pathology	Adenocarcinoma	5	(100)
	Mucinous adenocarcinoma	0	(0)
Grade	Moderate	5	(100)
	Poor	0	(0)
Mucinous component	Yes	0	(0)
	No	5	(100)
Signet cell component	Yes	0	(0)
	No	5	(100)
LVSI	Yes	1	(20)
	No	0	(0)
PNI	Yes	2	(40)
	No	3	(60)
Microsatellite status	MSS	5	(100)
	MSI-H	0	(0)
RAS	Wild-type	2	(40)
	Mutation	3	(60)
BRAF	Wild-type	5	(100)
	Mutation	0	(0)

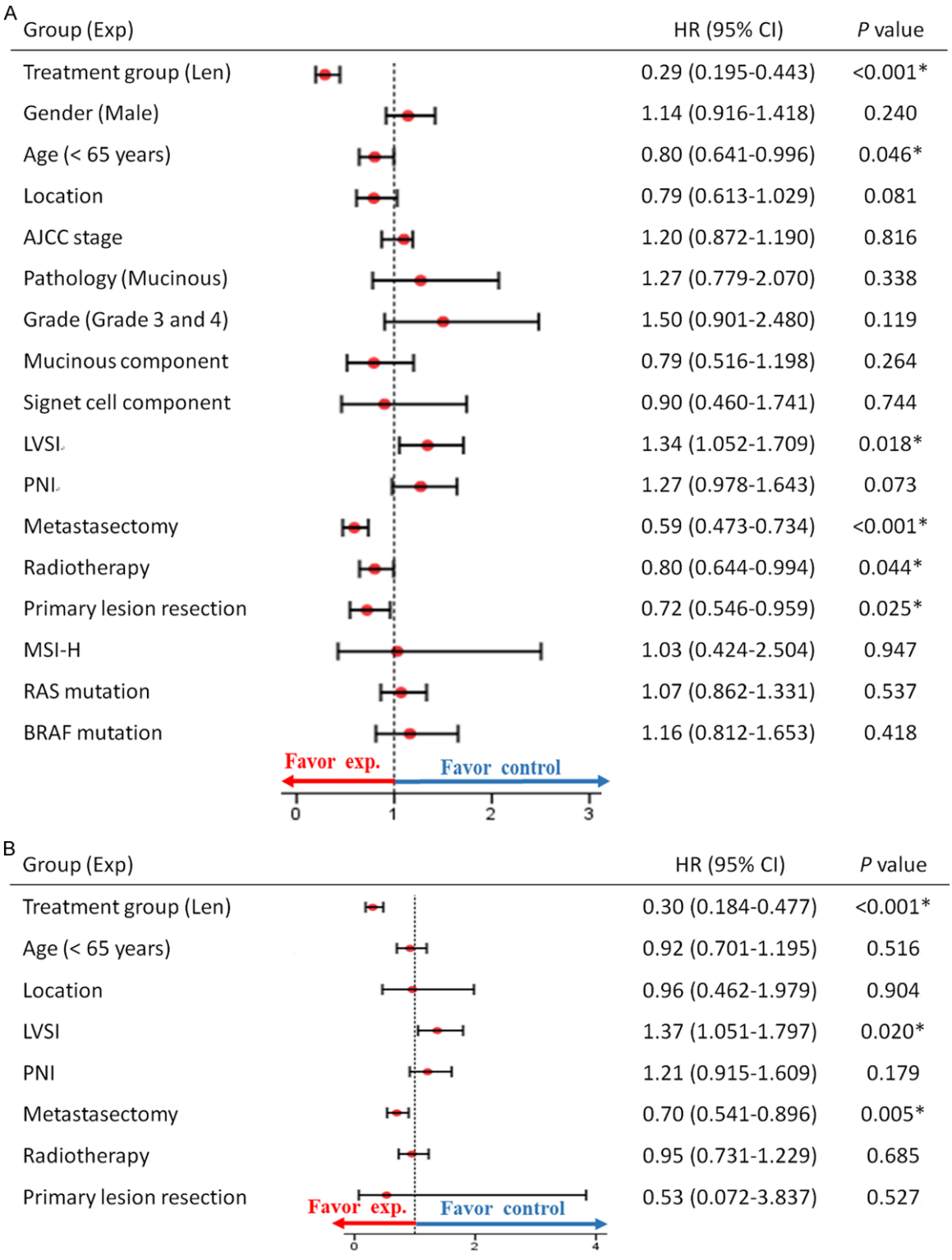
Abbreviations: AJCC, American Joint Committee on Cancer staging system; LVSI, lymphovascular invasion; PNI, perineural invasion; MSS, Microsatellite Stable; MSI-H, Microsatellite Instability-High.



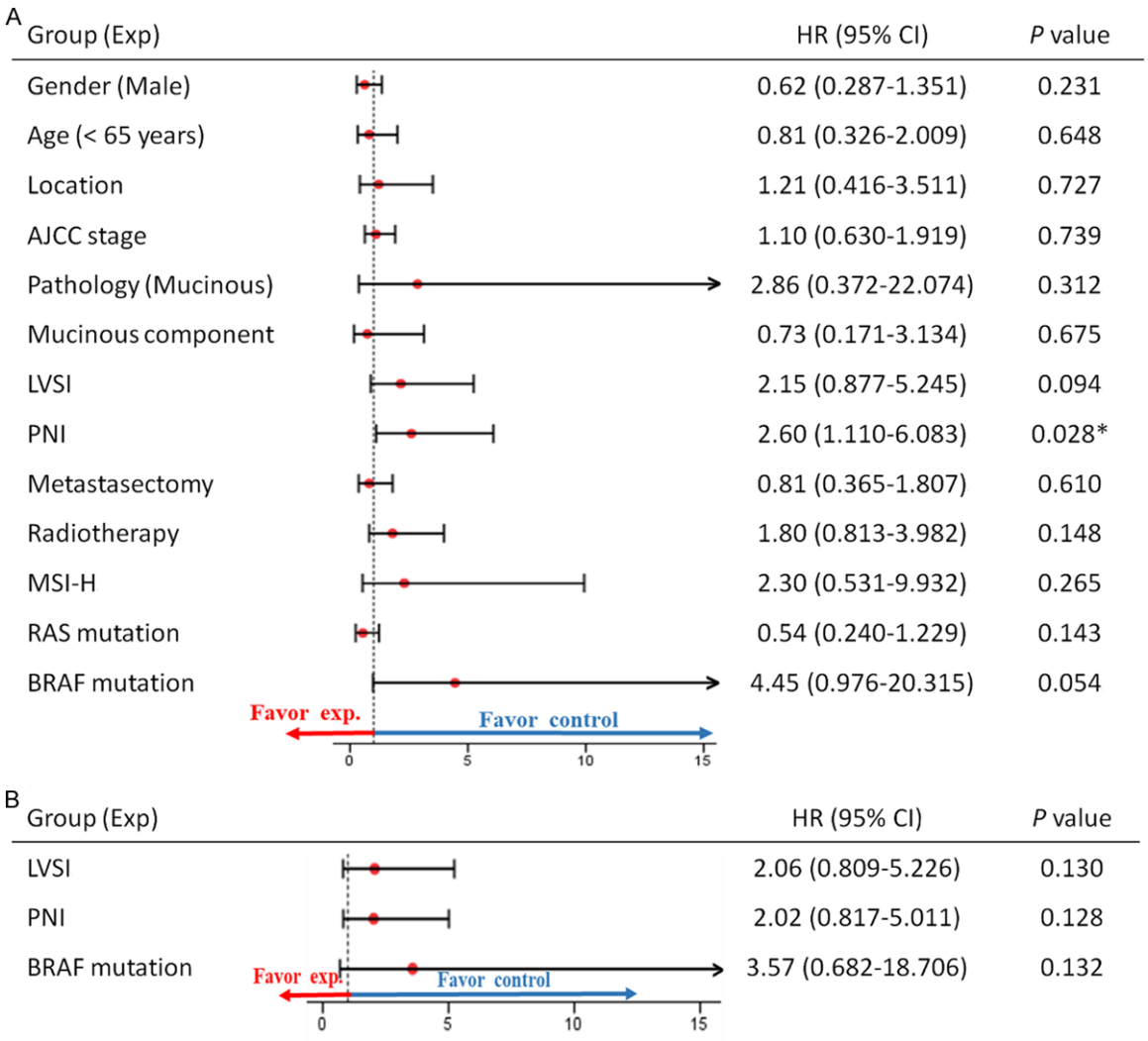
Supplementary Figure 2. OS1 of salvage lenvatinib-based group by combining C/T or IO. Abbreviations: Len, lenvatinib; OS, overall survival; C/T, chemotherapy; IO, immunotherapy.



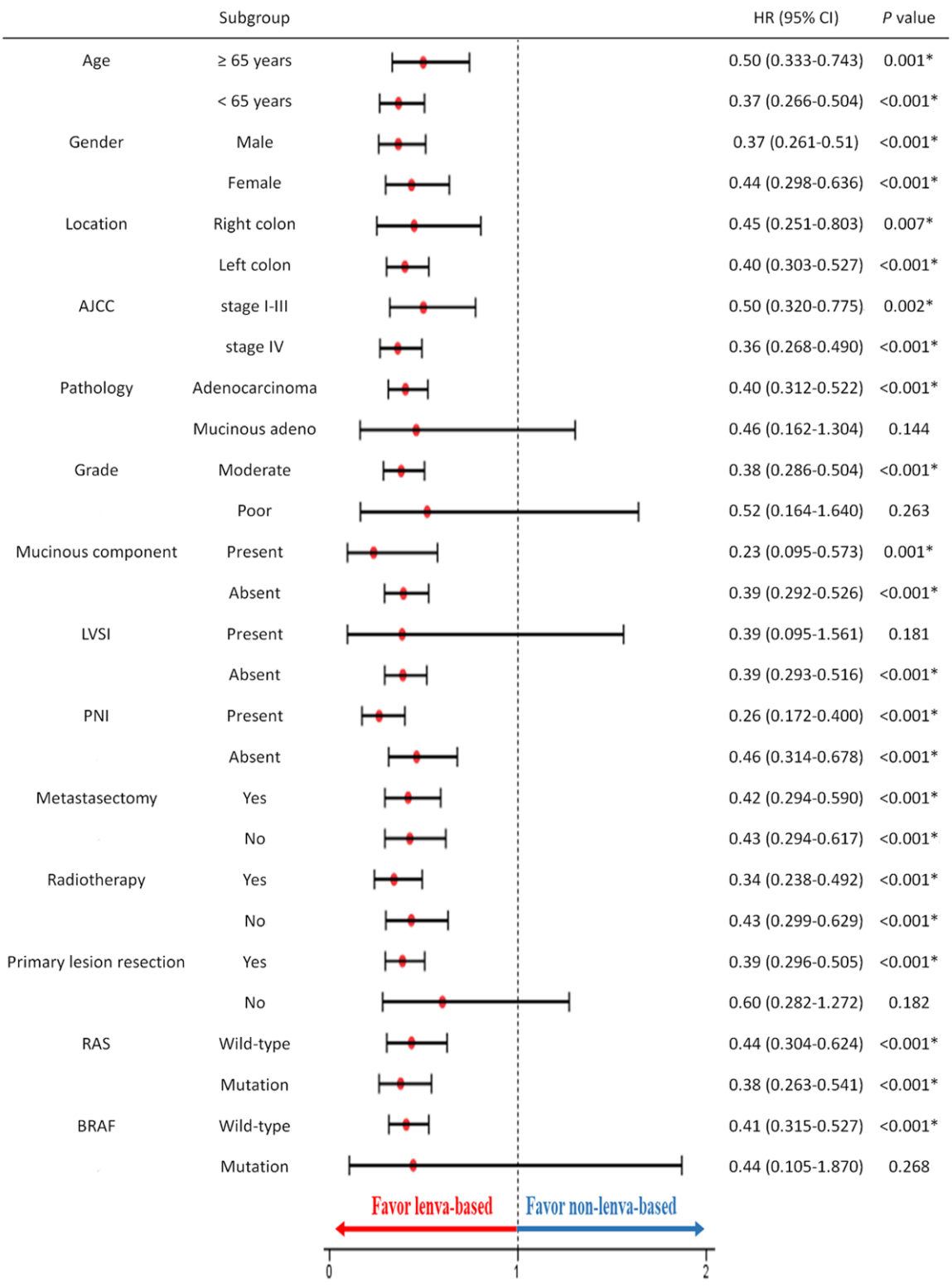
Supplementary Figure 3. Antitumor response in patients with salvage lenvatinib-based regimen and lenvatinib prior to receiving TAS-102 or regorafenib.



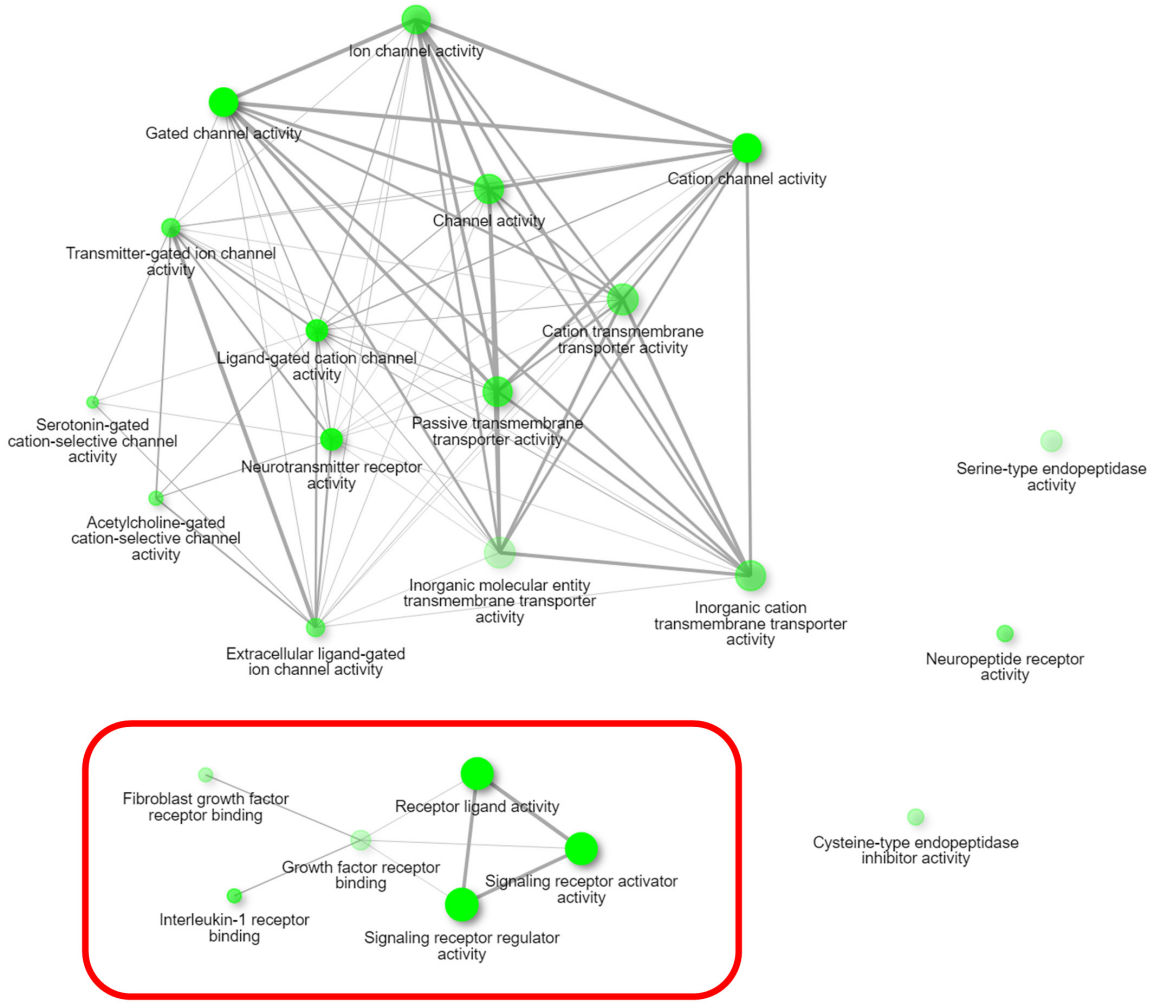
Supplementary Figure 4. Exploring clinical-pathological factors impacting OS2 in mCRC patients. A. Univariate analysis of OS2 impacting factors. B. Multivariate analysis of OS2 impacting factors. Abbreviations: Len, lenvatinib; Exp, experimental group; AJCC, American Joint Committee on Cancer staging system; LVSI, lymphovascular invasion; PNI, perineural invasion; MSI-H, Microsatellite Instability-High; HR, hazard ratio. *P value < 0.05.



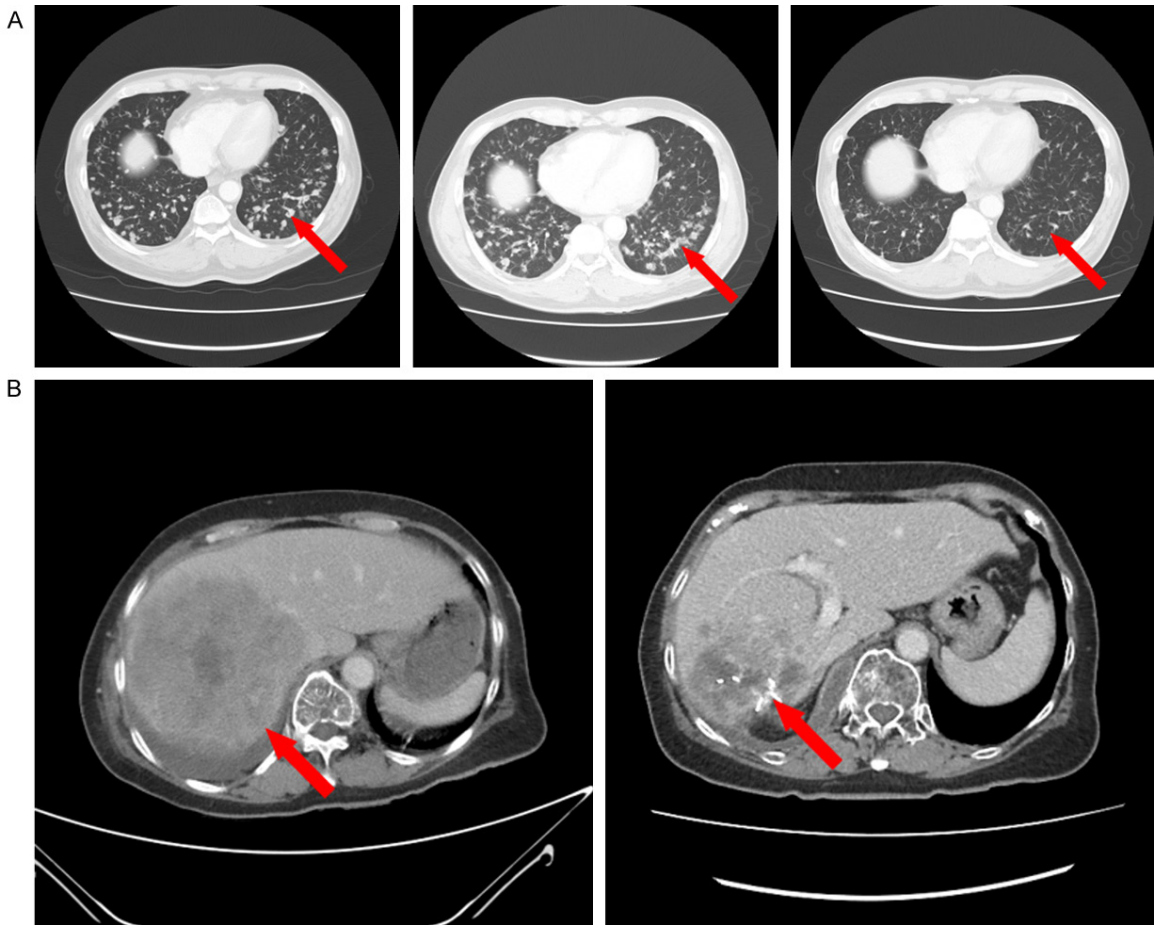
Supplementary Figure 5. Exploring clinical-pathological factors impacting OS1 in patients with salvage lenvatinib-based regimen. A. Univariate analysis of OS1 impacting factors. B. Multivariate analysis of OS1 impacting factors. Abbreviations: Exp, experimental group; AJCC, American Joint Committee on Cancer staging system; LVSI, lympho-vascular invasion; PNI, perineural invasion; MSI-H, Microsatellite Instability-High; HR, hazard ratio. *P value < 0.05.



Supplementary Figure 6. Subgroup analysis of OS2 in mCRC patients. Abbreviations: AJCC, American Joint Committee on Cancer staging system; LVSI, lymphovascular invasion; PNI, perineural invasion; HR, hazard ratio. *P value < 0.05.



Supplementary Figure 7. The network analysis of significant pathways TCGA database.



Supplementary Figure 8. Representative case with regressive change in metastatic target lesions after salvage lenvatinib-based regimen. A. 0 month, 2.3 month, 7.8 month. B. 0 month, 3.7 month.

Supplementary Table 2. Comparison between LEMON, LEAP-005, LEAP-017 studies

	LEMON	LEAP-005	LEAP-017
Type and design	Single center single arm phase 2	Multicenter multicohort phase 2	Multicenter randomized phase 3
Sample size	30	32 (in CRC cohort)	480 (241 vs 239)
Study populations	mCRC patient refractory to at least 2 lines of standard therapies ± TAS-102	mCRC patient refractory to at least 2 lines of standard therapies	mCRC patient refractory to at least 2 lines of standard therapies
Experimental group	Lenvatinib 24 mg QD	Lenvatinib 20 mg QD + Pembrolizumab 200 mg Q3W	Lenvatinib 20 mg QD + Pembrolizumab 400 mg Q6W
Control group	Nil	Nil	Regorafenib, TAS-102
Primary outcome	DCR	ORR, AE	OS
Secondary outcome	PFS, OS, ORR, AE	PFS, OS, DCR, DOR	PFS, ORR, DOR, AE, QOL
Main result	DCR 70% PFS 3.6 (2.6-3.7) mos OS 7.4 (6.4-10.8) mos ORR 6.7%	ORR 22% PFS 2.3 (2.0-5.2) mos OS 7.5 (3.9-NR) mos DCR 47% DOR NR	OS 9.8 (8.4-11.6) vs 9.3 (8.2-10.9) mos PFS 3.8 (3.7-5.1) vs 3.3 (2.0-3.7) mos ORR 10.4 vs 1.7% DCR 63.1 vs 52.7% DOR 11.1 vs 7.6 mos

Abbreviations: CRC, colorectal cancer; OS, overall survival; PFS, progression free survival; ORR, objective response rate; DOR, duration of response; DCR, disease control rate; AE, adverse events; QOL, quality of life; NR, not reached.