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RESEARCH

Fruquintinib plus sintilimab in previously bevacizumab-treated, pMMR/MSS refractory metastatic colorectal cancer: a phase 2 clinical trial

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Abstract

Background This study aimed to investigate the efficacy and safety of fruquintinib plus sintilimab in mismatch repair-proficient (pMMR)/microstatellite stable (MSS) refractory metastatic colorectal cancer (mCRC).

Methods Patients with pMMR/MSS mCRCs who had failed at least 2 lines of standard therapy were enrolled and treated with fruquintinib plus sintilimab in this single arm, phase II clinical trial. The primary endpoint was the 6 month progression-free survival (PFS) rate.

Results A total of 75 patients were included, all of whom had been previously treated with bevacizumab. The 6 month PFS rate was 33.3%. The median PFS (mPFS) was 4.1 months, the median overall survival (mOS) was 15.3 months, the objective response rate (ORR) was 12.5%, and the disease control rate (DCR) was 76.4%. Treatment-related adverse events (TRAEs) occurred in 94.7%, with 18.7% being grade 3 or 4. There were no treatment-related deaths. Patients had a significantly shorter mPFS compared to those without liver metastasis (3.2 versus 7.6 months, $P<0.001$). Incorporating Eastern Cooperative Oncology Group (ECOG) score further improved its predictive value for PFS. Those in the high-albumin group showed significantly longer mOS (25.9 versus 11.0 months, $P=0.024$), while the high-neutrophil-to-lymphocyte ratio (NLR) group exhibited a significantly shorter mOS (9.3 versus 19.3 months, $P=0.033$).

Conclusions Fruquintinib plus sintilimab showed promising activity and good tolerability in refractory pMMR/MSS mCRC. Liver metastasis was a negative predictor for efficacy and PFS, especially when combined with the ECOG score. Higher baseline albumin and lower NLR predicted longer OS.

Keywords Colorectal cancer · Immune checkpoint inhibitor · Tyrosine kinase inhibitor · Liver metastasis · Neutrophil-to-lymphocyte ratio · Albumin

Introduction

Colorectal cancer (CRC) is the third most common cancer and the second most deadly cancer worldwide [1]. In China, colorectal cancer ranks as the fourth most common malignancy and accounted for approximately 240,000 deaths in 2022 [2]. In recent years, systemic chemotherapy combined with targeted therapy has significantly prolonged the survival times of metastatic CRC (mCRC) patients. Effective treatment options remain limited for mCRC patients who have failed standard therapies. The main standard later-line treatments, including trifluridine-tipiracil (alone or with bevacizumab), regorafenib, and fruquintinib, show ORRs below 6%, with PFS of 2–5.5 months and OS of 7.1–10.8 months [3–6].

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Immune checkpoint inhibitors (ICIs) have demonstrated significant efficacy in patients with mismatch repair-deficient/microsatellite instability-high (dMMR/MSI-H) mCRC. However, their effectiveness is markedly limited in mismatch repair-proficient/microsatellite stable (pMMR/MSS) mCRC, which constitutes approximately 95% of all mCRC cases. The limited response in pMMR/MSS tumors is thought to be associated with a low tumor mutational burden (TMB), reduced neoantigen load, and a paucity of tumor-infiltrating lymphocytes [7, 8].

Many studies have investigated ICI-based combination therapies to improve immunotherapy responses in pMMR/MSS colorectal cancer. The phase Ib REGONIVO study evaluating regorafenib in combination with nivolumab reported an ORR of 33% in patients with pMMR/MSS mCRC [9], which marked the beginning of exploring the combination of anti-angiogenic tyrosine kinase inhibitors (TKIs) and ICIs. Several subsequent studies explored this regime for the treatment of pMMR/MSS mCRC, and the ORRs varied widely, ranging from 7 to 15.2%, with the mOS ranging from 11.1 to 15.2 months [10–12]. However, the phase III randomized trial LEAP-017 demonstrated that lenvatinib combined with pembrolizumab did not achieve a significant OS benefit compared to regorafenib or trifluridine-tipiracil in MSS mCRC patients [13]. The clinical value of TKI-ICI combination regimens in advanced colorectal cancer remains a subject of debate. Nevertheless, the LEAP-017 study yielded some positive findings in subgroup analyses. A favorable OS benefit with lenvatinib plus pembrolizumab was observed in patients from Asia and in those without liver metastases, which suggest that future research should focus on identifying potential subpopulations who may derive the clinical benefit from this combination regimen.

Sintilimab is a programmed cell death protein 1 (PD-1) inhibitor approved in China, and fruquintinib is a highly selective small-molecule tyrosine kinase inhibitor (TKI) of vascular endothelial growth factor receptor (VEGFR) 1/2/3. In mouse models with MC38 or CT26 xenograft tumors of CRC, the combination of fruquintinib and sintilimab resulted in greater inhibition of tumor growth and a longer survival time than did fruquintinib or sintilimab alone [14], suggesting the potential value of combination therapy. Furthermore, bevacizumab-containing regimens are commonly used in first- or second-line treatment of mCRC. In later-line settings, patients previously treated with bevacizumab often exhibit diminished efficacy when rechallenged with the same agent [15–17]. However, a subgroup analysis of the phase III CONCUR trial showed that patients who had received prior anti-VEGF therapy did not benefit from regorafenib in terms of OS [18].

We conducted this phase 2 clinical trial to evaluate the efficacy and safety of fruquintinib in combination with sintilimab in previously bevacizumab-treated patients with pMMR/MSS mCRC who had failed standard therapies, and to explore potential subpopulations that may derive clinical benefit.

Methods

Study design and patient eligibility

This open-label, single-arm, single-center phase 2 study was conducted at the Cancer Hospital of the Chinese Academy of Medical Sciences, carried out in accordance with the principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and local applicable regulatory requirements, and approved by the Ethics Committee of the Cancer Hospital, Chinese Academy of Medical Sciences (Approval No. 19/208–1992). All patients provided written informed consent before enrollment. The study was registered at ClinicalTrials.gov (Registration No. NCT04695470).

The inclusion criteria for patients were as follows: age 18–75 years old; diagnosed with pathologically confirmed mCRC with an ECOG performance status of 0 or 1; pMMR/MSS/MSI-L classification; failure to respond to standard therapy, including fluorouracil (FU), oxaliplatin, irinotecan, bevacizumab, and cetuximab (for patients with Rat Sarcoma viral oncogene homolog (RAS) wild-type left-sided mCRC); and at least one measurable target lesion according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1). Patients with prior use of anti-angiogenic TKIs and ICIs were not eligible for enrollment.

Drug administration

The enrolled patients were orally treated with 5 mg of fruquintinib once daily for 2 weeks on and 1 week off in combination with 200 mg of sintilimab on day 1 and every 3 weeks thereafter. Treatment was continued until disease progression (PD) or the occurrence of unacceptable toxicity. Fruquintinib was permitted to be administered at a reduced dose according to the treatment-related adverse events (TRAEs), with the first dose tapered down to 4 mg/d and the second to 3 mg/d. A dose increase or decrease was not recommended for sintilimab, but it could be suspended or permanently discontinued at the discretion of the presiding clinicians. Patients with interruption or discontinuation of either fruquintinib or sintilimab therapy due to TRAEs were permitted to continue fruquintinib or sintilimab monotherapy until PD or unacceptable toxicity.

Endpoints

The primary endpoint was the 6-month progression-free survival (PFS) rate, and the secondary endpoints included the

objective response rate (ORR), disease control rate (DCR), clinical benefit rate (CBR; defined as the proportion of patients with a complete response [CR], a partial response [PR], and stable disease [SD] persisting for ≥ 24 weeks), progression-free survival (PFS), and overall survival (OS). Radiological assessments were performed with enhanced computed tomography (CT) or magnetic resonance imaging (MRI) according to RECIST v1.1 every 6 weeks (every 12 weeks after 48 weeks). The first occurrence of complete response (CR) or partial response (PR) was confirmed by re-evaluation at least 4 weeks later. The incidence and severity of adverse events (AEs) were graded in accordance with the Common Terminology Criteria for AEs version 5.0 (CTCAE 5.0).

Sample size

For patients with advanced colorectal cancer who have previously failed treatment with irinotecan, oxaliplatin, fluoropyrimidines, bevacizumab, and cetuximab (in RAS wild-type cases), subgroup analysis from the FRESCO study indicates a 6-month progression-free survival (PFS) rate of approximately 20%. It is hypothesized that treatment with sintilimab combined with fruquintinib may improve the 6 month PFS rate to 35%. Assuming a power of 80% and a two-sided alpha level of 0.05, a total of 63 patients are required to detect this difference. Considering a 10% dropout rate, the final sample size is set at 70 patients.

Statistical analysis

Statistical analysis was performed with the R 4.4.3 software (R Foundation for Statistical Computing, Vienna, Austria). The continuous variables were compared using the student's t-test or Wilcoxon rank-sum test. The categorical data were compared using the chi-square test or fisher's exact test. Median PFS and OS were calculated using the Kaplan-Meier method and displayed as median (95% confidence interval [CI]). The duration of follow-up was calculated using the reverse Kaplan-Meier estimate of OS. Survival curves were generated using the Kaplan-Meier method and compared using the log-rank test. For the analysis of biomarkers, 6 month PFS rate was the primary indicator, and PFS and OS were the secondary indicators. Univariate and multivariate Cox proportional hazards regression model survival analyses were performed to identify prognostic factors. Receiver operating characteristic (ROC) curves were generated for each selected factor, and the optimal cut-off value that maximized the Youden index was identified for subsequent grouping and analyses. Statistical tests were two-sided, and a P value < 0.05 was considered significant.

Results

Patient characteristics

From 2020/9/18 to 2022/10/28, 113 patients were screened for the study, and 75 patients were enrolled (Fig. 1). Enrollment was slightly expanded to ensure an adequate number of patients for the planned analyses after screening. The demographic and clinical characteristics of the patients are presented in Table 1. The median age was 57 years (Interquartile range [IQR]: 52–65), and 66.7% (50/75) of the patients were male. The primary lesion occurred in the left colon or rectum in 84.0% (63/75) of patients. A total of 61.3% (46/75) of patients had an ECOG score of 1. The proportions of patients with lung and liver metastasis were 64.0% (48/75) and 60.0% (45/75), respectively. 30.7% (23/75) of patients had received ≥ 3 prior lines of therapy, and all had been previously treated with bevacizumab.

Efficacy

The median number of treatment cycles was 5 (range, 1–31). The median time from first-line therapy to study entry was 22.2 months (IQR, 16.3–32.9). The mPFS was

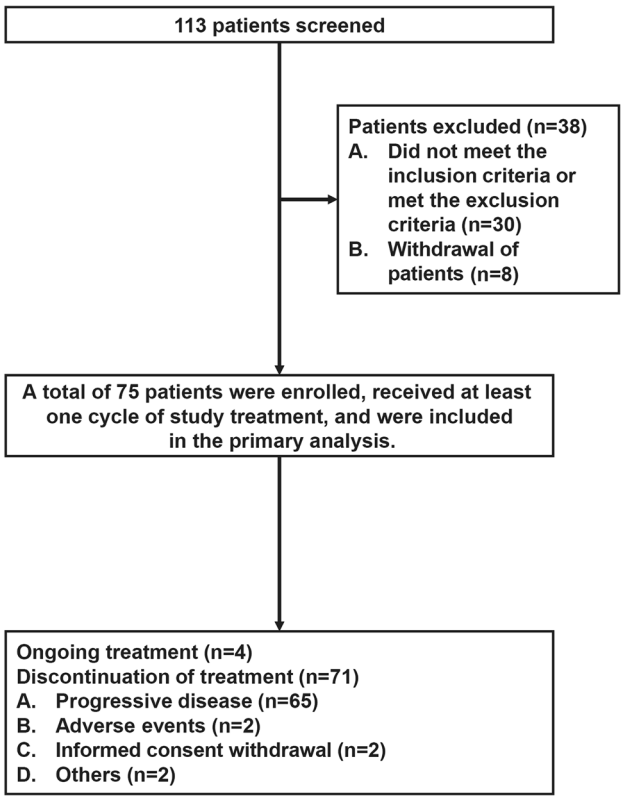

Fig. 1 Study flowchart of the entire population

Table 1 Baseline characteristics and survival analyses

Characteristics	All patients (n = 75)	Median PFS, months (95% CI)	P value (log rank)	Median OS, months (95% CI)	P value (log rank)
Sex, n (%)					
Male	50 (66.7%)	4.8 (3.7, 7.7)	0.006	15.3 (8.1, NA)	0.984
Female	25 (33.4%)	3.0 (2.7, 4.7)		13.3 (8.8, NA)	
Age, years					
Median (IQR)	57 (52–65)				
≥ 60, n (%)	44 (58.67%)	3.7 (2.1, 7.7)	0.403	11.0 (8.0, NA)	0.898
< 60, n (%)	31 (41.33%)	4.5 (4.0, 6.4)		15.4 (9.1, NA)	
ECOG performance status, n (%)					
0	29 (38.7%)	6.4 (4.8, 8.4)	< 0.001	19.5 (16.1, NA)	< 0.001
1	46 (61.3%)	3.3 (2.7, 4.3)		8.1 (7.0, 15.4)	
Site of primary tumor, n (%)					
Left colon and rectum	63 (84.0%)	4.1 (3.0, 5.9)	0.780	15.3 (9.1, 19.5)	0.447
Right colon	12 (16.0%)	4.0 (2.8, NA)		15.6 (7.8, NA)	
Time from first-line therapy to study entry, n (%)					
≥ 18 months	50 (66.7%)	5.7 (4.1, 7.7)	0.002	16.2 (13.3, NA)	0.016
< 18 months	25 (33.3%)	3.0 (2.7, 4.1)		9.1 (5.7, 15.6)	
Number of metastatic sites, n (%)					
1	18 (24.0%)	7.8 (5.8, 14.2)	0.003	19.3 (15.3, NA)	0.039
> 1	57 (76.0%)	3.7 (2.7, 4.3)		10.8 (8.0, 16.2)	
Liver metastasis, n (%)					
Yes	45 (60.0%)	3.2 (2.7, 4.1)	< 0.001	10.0 (7.8, 19.3)	0.138
No	30 (40.0%)	7.6 (5.8, 9.1)		15.5 (13.3, NA)	
Lung metastasis, n (%)					
Yes	48 (64.0%)	4.1 (3.0, 5.9)	0.335	13.3 (8.0, 19.5)	0.297
No	27 (36.0%)	4.1 (2.9, 8.3)		15.4 (9.1, NA)	
Prior treatment line, n (%)					
2nd line	52 (69.3%)	4.4 (3.2, 6.8)	0.248	15.4 (8.1, 27.0)	0.920
3rd line +	23 (30.7%)	4.0 (2.7, 6.9)		11.0 (9.1, NA)	
Previous treatment, n (%)					
Prior anti-VEGF inhibitors	75 (100.0%)	4.1 (3.2, 5.8)	NA	15.3 (9.3, 19.3)	NA
No prior anti-VEGF inhibitors	0 (0.0%)	NA		NA	
Prior anti-EGFR inhibitors	21 (28.0%)	4.1 (2.7, 6.8)	0.346	15.3 (8.8, NA)	0.783
No prior anti-EGFR inhibitors	54 (72.0%)	4.1 (3.0, 6.9)		15.3 (8.1, 25.9)	
Excision of primary lesion, n (%)					
Yes	62 (82.7%)	4.4 (4.0, 6.9)	< 0.001	15.3 (9.3, 27.0)	0.091
No	13 (17.3%)	2.7 (1.6, NA)		10.8 (3.1, NA)	
RAS/BRAF^{V600E} status, n (%)					
RAS mutant	49 (65.3%)	4.1 (2.9, 5.8)	0.845	13.3 (8.8, 25.9)	0.913
RAS wild-type	26 (34.7%)	4.4 (2.8, 7.8)		15.6 (7.5, NA)	
BRAF^{V600E} mutant	0 (0.0%)	NA	NA	NA	NA
BRAF^{V600E} wild-type	75 (100.0%)	4.1 (3.2, 5.8)		15.3 (9.3, 19.3)	

PFS, progression-free survival; *OS*, overall survival; *P* value, probability value; *IQR*, interquartile range; *VEGF*, vascular endothelial growth factor; *RAS*, rat sarcoma viral oncogene homolog; *BRAF*, b-raf proto-oncogene, serine/threonine kinase; *ECOG*, Eastern Cooperative Oncology Group; *EGFR*, epidermal growth factor receptor

4.1 months (95% CI, 3.2–5.8 months), and the mOS was 15.3 months (95% CI, 9.3–19.3 months) (Fig. 2a, b). 72 patients underwent at least 1 response evaluation. 9 and 46 patients achieved partial response (PR) and stable disease (SD), respectively. Typically, the ORR and DCR were calculated in the evaluable population (n = 72), whereas the intention-to-treat (ITT) population (n = 75) was used for endpoints such as the 6-month PFS rate and

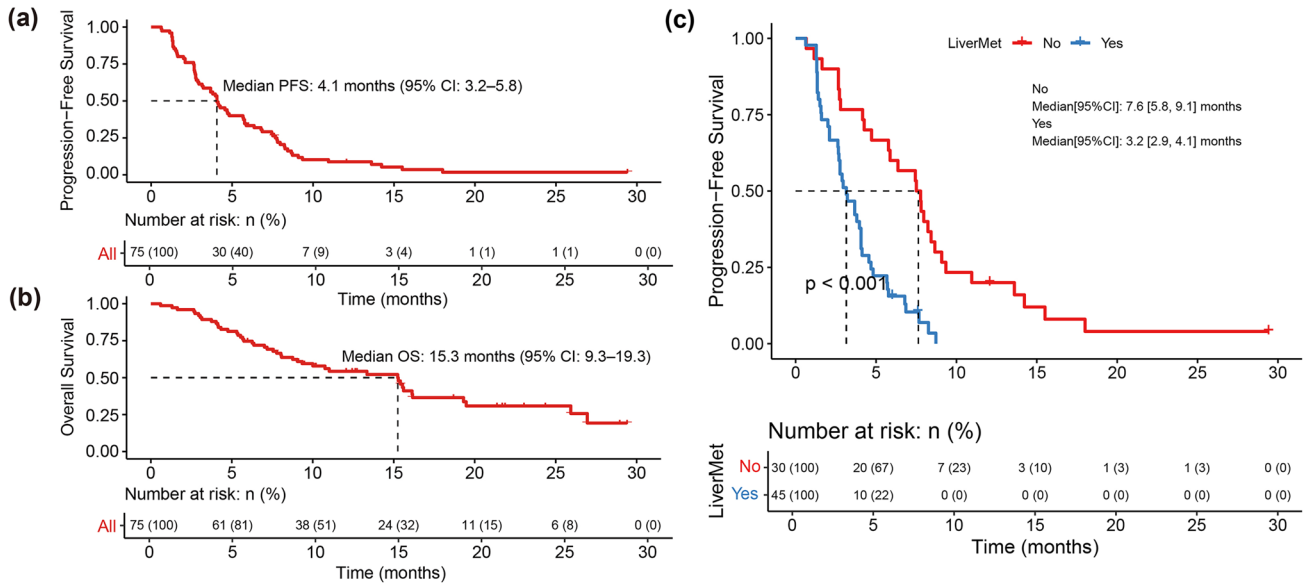

Fig. 2 Survival outcomes and prognostic impact of liver metastasis in mCRC patients receiving combination therapy **a** Kaplan-Meier estimate of PFS in all patients; **b** Kaplan-Meier estimate of OS in all patients; **c** Kaplan-Meier curve for PFS stratified by liver metastasis

Table 2 Efficacy outcomes

Variable[#]	n (%)
Confirmed best response	
CR	0 (0.0)
PR	9 (12.5)
SD	46 (63.9)
PD	17 (23.6)
ORR, % (95% CI)	12.5 (5.9, 22.4)
DCR, % (95% CI)	76.4 (64.9, 85.6)
6-month PFS rate, % (95% CI)	33.3 (22.9, 45.2)
CBR, % (95% CI)	37.3 (26.4, 49.3)
Median PFS (95% CI), months	4.1 (3.2, 5.8)
Median OS (95% CI), months	15.3 (9.3, 19.3)

[#] Confirmed best response, ORR, and DCR were evaluated in the evaluable population (n = 72), whereas the intention-to-treat (ITT) population (n = 75) was used for endpoints including the 6 month PFS rate, CBR, median PFS, and median OS

CR, complete response; *PR*, partial response; *SD*, stable disease; *PD*, progressive disease; *PFS*, progression-free survival; *CI*, confidence interval; *ORR*, objective response rate; *DCR*, disease control rate; *CBR*, clinical benefit rate; *DoR*, duration of response; *OS*, overall survival

the CBR. The results showed an ORR of 12.5% (9/72, 95% confidence interval [CI], 5.9–22.4%), a CBR of 37.3% (28/75, 95% CI, 26.4–49.3%), and a DCR of 76.4% (55/72, 95% CI, 64.9–85.6%). The primary endpoint of the 6 month PFS rate was 33.3% (25/75, 95% CI, 22.9–45.2%) (Table 2).

Safety

Among the 75 enrolled patients, TRAEs occurred in 94.7% (71/75), with 18.7% (14/75) being grade 3 or 4. There were no treatment-related deaths. The most common AEs (incidence ≥ 15%) were proteinuria (48.0%, 36/75), hypothyroidism (49.3%, 37/75), hypertension (32.0%, 24/75), hand−foot skin reactions (28.0%, 21/75), dysphonia (20.0%, 15/75), and elevated transaminase level (20.0%, 15/75). The grade 3 TRAEs (incidence ≥ 5%) were hand−foot skin reactions (9.3%, 7/75) and fatigue (5.3%, 4/75). Two patients experienced grade 4 TRAEs, including one case of hypertension and one case of diarrhea. The incidence of immune-related adverse events (irAEs) was 53.3% (40/75), most of which were grades 1–2. Grade 3 irAEs occurred in 8.0% (6/75) of the patients, including fever (2.7%, 2/75), hyperthyroidism (1.3%, 1/75), elevated blood creatinine (1.3%, 1/75), immune-related pneumonia (1.3%, 1/75), and immune-related liver disease (1.3%, 1/75). No grade 4 irAEs were observed. The details of the adverse events (AEs) are shown in Table 3. Five patients (5/75, 6.7%) underwent fruquintinib dose reduction due to AEs. The dose was reduced to 4 mg/d in 4 patients and to 3 mg/d in 1 patient. 2 patients discontinued treatment due to adverse events, and another 2 experienced treatment interruptions related to Coronavirus Disease 2019 (COVID-19).

Poorer efficacy in patients with liver metastasis

Survival analysis revealed that patients with liver metastasis had a significantly shorter mPFS compared to those

Table 3 Summary of adverse events

Adverse events	Grade 1–2, n (%)	Grade 3, n (%)	Grade 4, n (%)	Total, n (%)
Total	57 (76.0)	12 (16.0)	2 (2.7)	71 (94.7)
Immune-related adverse events	34 (45.3)	6 (8.0)	0	40 (53.3)
Proteinuria	36 (48.0)	2 (2.7)	0	38 (50.7)
Hypothyroidism	37 (49.3)	0	0	37 (49.3)
Hand-foot skin reaction	21 (28.0)	7 (9.3)	0	28 (37.3)
Hypertension	24 (32.0)	0	1 (1.3)	25 (33.3)
Dysphonia	15 (20.0)	0	0	15 (20.0)
Elevated transaminase levels	15 (20.0)	0	0	15 (20.0)
Diarrhea	10 (13.9)	3 (4.0)	1 (1.3)	14 (18.7)
Fatigue	8 (10.7)	4 (5.3)	0	12 (16.0)
Decreased albumin levels	11 (14.7)	0	0	11 (14.7)
Anorexia	10 (13.3)	1 (1.3)	0	11 (14.7)
Elevated bilirubin levels	7 (9.3)	1 (1.3)	0	8 (10.7)
Fever	5 (6.7)	2 (2.7)	0	7 (9.3)
Anemia	7 (9.3)	0	0	7 (9.3)
Neutropenia	4 (5.3)	0	0	4 (5.3)
Rash	3 (4.0)	0	0	3 (4.0)
Thrombocytopenia	3 (4.0)	0	0	3 (4.0)
Myalgia	2 (2.7)	0	0	2 (2.7)
Hyperthyroidism	0	1 (1.3)	0	1 (1.3)
Elevated blood creatinine levels	0	1 (1.3)	0	1 (1.3)
Pneumonia	0	1 (1.3)	0	1 (1.3)

without liver metastasis (3.2 versus 7.6 months, $P < 0.001$) (Fig. 2c). Consistently, univariate Cox regression analysis also demonstrated a significant association between liver metastasis and shorter PFS (hazard ratio [HR] [95%CI], 3.40 [1.94–5.97], $P < 0.001$). In addition, patients with liver metastasis exhibited a significantly lower 6-month PFS rate (15.6% versus 60.0%, $P < 0.001$). The ORR (2.3% versus 27.6%, $P = 0.002$), CBR (17.8% versus 66.7%, $P < 0.001$), and DCR (67.4% versus 89.7%, $P = 0.046$) were also markedly reduced in this subgroup (Table S1).

Subsequently, we performed variable selection using least absolute shrinkage and selection operator (LASSO) regression based on the following criteria: (1) variables with $P < 0.10$ in log-rank test of baseline characteristics; (2) variables with $P < 0.10$ in univariate Cox analysis (Fig. S1a); and (3) essential clinicopathological factors. Due to the substantial missing data on immunoglobulin subclasses, these variables were excluded from subsequent analyses. A multivariable stepwise regression model was subsequently applied to identify the optimal predictive model among the 70 patients with available data.

The final multivariate Cox regression model further revealed that liver metastasis (HR [95%CI], 3.38 [1.87–6.12], $P < 0.001$) was an independent predictor of PFS, along with ECOG performance status of 1 (HR [95%CI], 2.23 [1.30–3.82], $P = 0.004$) (Fig. S1b). A nomogram was constructed to visualize the predictive capacity of these two factors for PFS at various time points (Fig. S1c).

Based on the combination of liver metastasis and ECOG score, patients receiving fruquintinib plus sintilimab were stratified into three prognostic risk groups: the high-risk group (patients with liver metastasis and an ECOG score of 1), the intermediate-risk group (patients with no liver metastasis but an ECOG score of 1, or with liver metastasis but an ECOG score of 0), and the low-risk group (patients without liver metastasis and an ECOG score of 0), with the mPFS showing a clear gradient among the three groups (2.8 versus 4.8 versus 8.2 months, $P < 0.001$) (Fig. S1d). The predictive value for PFS was significantly improved when combining the two factors compared to using either factor alone (Fig. S1e).

Elevated baseline albumin predicts better overall survival

We also performed LASSO regression for variable selection based on the following criteria: (1) variables with $P < 0.01$ in log-rank test of baseline characteristics; (2) variables with $P < 0.10$ in the univariate Cox analysis for OS (Fig. S2a); and (3) essential clinicopathological factors. Due to the substantial missing data on immunoglobulin subclasses, these variables were excluded from subsequent analyses. A multivariable stepwise regression model was subsequently

applied to identify the optimal predictive model among the 70 patients with available data.

The final multivariate Cox regression model included four factors: carcinoembryonic antigen (CEA), ECOG score, baseline NLR, and baseline albumin (Fig. S2b), which further revealed that elevated baseline albumin (HR [95%CI], 0.91 [0.82–0.99], $p = 0.037$) and ECOG performance status of 1 (HR [95%CI], 2.99 [1.38–6.48], $p = 0.006$) were independent predictors of OS. When patients were stratified according to the optimal cut-off values of baseline baseline albumin, CEA, and NLR, those in the high-albumin group showed significantly longer mOS (25.9 versus 11.0 months, $P = 0.024$), while the high-CEA group (8.8 versus 19.3 months, $P = 0.009$) and high-NLR group (9.3 versus 19.3 months, $P = 0.033$) exhibited a significantly shorter mOS (Fig. S2c–f).

Discussion

Our study enrolled patients with MSS mCRC who had all received prior treatment with bevacizumab. Additionally, all patients with RAS wild-type left-sided tumors had previously been treated with cetuximab. The primary endpoint of 6-month PFS rate was 33.3%. with an ORR of 12.5%, a CBR of 37.3%, and a DCR of 76.4%. The mPFS was 4.1 months, and the mOS was 15.3 months. These results seem to be more promising than those of TKI treatment alone. In the CORRECT study[19], which included the same population, the ORR with regorafenib monotherapy was only 1%. The mPFS and mOS were 1.9 and 6.4 months, respectively. In the subgroup analysis of the FRESCO trial, the ORR for fruquintinib treatment was 3.6%, with mPFS and mOS of 3.5 and 7.2 months, in patients who previously received anti-VEGF therapy [20], respectively. The results of our study are similar to other studies of combination therapies that have been reported to date[21–25], especially in patients previously treated with bevacizumab. The safety profile was favorable, with an incidence of grade 3–4 TRAEs of 18.67%.

Given that the LEAP-017 study did not meet its primary endpoint, the clinical value of combining ICIs with TKIs for treating advanced MSS mCRC remains a subject of ongoing debate. However, it is undeniable that a subset of patients with MSS mCRC may still benefit from this combination strategy. Most patients experience a PFS benefit from this regimen, except for subgroups such as those aged ≥ 65, with an ECOG score of 1, or with RAS wild-type. Additionally, patients from Asia and those without liver metastases showed a favorable OS benefit when treated with lenvatinib plus pembrolizumab. Therefore, one of the key objectives of our study was to identify the specific subpopulations that could derive clinical benefit from this treatment approach.

Liver metastasis is a poor predictive factor for immunotherapy, as shown by the REGOTORI study, where the ORRs in patients with and without liver metastases were 8.7% and 30%, respectively [12]. In the phase 2 clinical study reported by Marwan Fakih, in which regorafenib combined with nivolumab was used as a later-line treatment for MSS mCRC, the ORR in patients with and without liver metastasis was 0% and 22%, respectively [10]. Our study further supported this conclusion. Patients with liver metastases failed to derive meaningful clinical benefit from immunotherapy. This may be attributed to both the inherently immunosuppressive microenvironment of the liver [26, 27] and the additional immunosuppression induced by liver metastases [28, 29], which together may hinder the synergistic effect of TKI–ICI combination therapy. Future research should focus on strategies to overcome the immunosuppressive microenvironment associated with liver metastases.

Commonly used indicators such as CEA and ECOG score were also found to be associated with prognosis in mCRC patients receiving ICI combined with anti-VEGF TKI, which aligns with the clinical rationale that patients with lower tumor burden and better overall condition are more likely to experience prolonged survival and benefit from treatment. Our study highlights the prognostic value of these indicators and supports their use in routine monitoring and treatment planning [30, 31].

Furthermore, we proposed a simple model incorporating ECOG score and liver metastasis to preliminarily predict mPFS in patients with mCRC receiving ICI plus TKI therapy. This model integrates both the patient's general condition and metastatic characteristics, providing a rapid, noninvasive, and easily applicable tool to assist in PFS stratification and guide clinical decision-making. However, given the limited sample size, this model requires further validation.

We also observed some positive findings regarding serum proteins, among which albumin, the major component of serum proteins, emerged as particularly relevant. Previous evidence suggests that serum albumin may serve as a prognostic biomarker in patients with advanced cancers receiving ICIs [32, 33]. A high pretreatment serum albumin level has been associated with favorable radiographic responses following ICI treatment [34]. Consistent with these findings, our study demonstrated that in patients with mCRC receiving combination therapy with ICIs and anti-VEGF TKIs, higher baseline albumin levels were significantly associated with prolonged OS. Low albumin may be associated with systemic inflammation and cachexia, contributing to an immunosuppressive tumor microenvironment [35–37]. As ICIs are immunoglobulin G (IgG)-based therapeutic antibodies, IgG and albumin both bind to the neonatal Fc receptor (FcRn) at distinct, non-cooperative, and non-competitive sites. Serum albumin levels, which reflect FcRn abundance

and functionality, may serve as a surrogate marker for the catabolic rate of therapeutic IgG antibodies, and potentially predict the efficacy of ICIs [38, 39]. Further studies are needed to clarify the pharmacokinetics between serum albumin and ICIs, and to assess whether correcting hypoalbuminemia could improve outcomes.

In colorectal cancer, the neutrophil-to-lymphocyte ratio (NLR) is an important inflammatory marker, and elevated NLR levels are often associated with poor survival outcomes [40], particularly in the context of liver metastases [41]. NLR correlates not only with more aggressive tumour characteristics but also with the intracellular distribution of programmed death-ligand 1 (PD-L1), which may reduce the targetability of ICIs [42]. Moreover, a high systemic NLR is associated with an increased presence of tumor-associated neutrophils (TANs), which may contribute to an immunosuppressive microenvironment that impairs the efficacy of ICIs [43]. In gastric cancer patients treated with ICIs, elevated NLR has been significantly correlated with shorter OS and PFS [44]. Similarly, in melanoma and non-small cell lung cancer, high baseline NLR has been reported as an independent negative prognostic factor for survival and is frequently associated with resistance to ICIs [45, 46]. These findings suggested that elevated NLR may compromise the effectiveness of immunotherapy. Consistent with prior studies [47], our findings demonstrate that baseline NLR is predictive of overall survival in patients treated with fruquintinib plus sintilimab, thereby reinforcing the prognostic relevance of NLR in colorectal cancer immunotherapy.

Our study has several limitations. First, the number of enrolled patients was relatively small, which may have limited the statistical power of the conclusions. Second, as a single-arm study, the evaluation of the efficacy and safety of fruquintinib plus sintilimab is limited by the study design and may be influenced by bias or confounding factors. Third, some hematological parameters were missing in a subset of patients, particularly immunoglobulin subclasses, therefore, the analyses involving these variables were exploratory, potentially subject to significant bias, and require further validation.

Conclusions

The combination of fruquintinib and sintilimab demonstrated promising antitumor activity and manageable safety in refractory pMMR/MSS mCRC. Liver metastasis, particularly when integrated with ECOG score, emerged as a strong negative predictor for treatment efficacy and PFS. Furthermore, higher baseline albumin and lower baseline NLR were associated with improved overall survival. These findings may help guide patient selection for ICI plus TKI therapy and warrant validation in prospective studies.

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Author contributions APZ and WZ conceived the project and designed the experiments. APZ, WZ, CFG, WQ, ZCJ, JLH and LY contributed to patient enrollment and related sample/data analysis. CFG and WZ conducted the experiments and acquired the data. CFG, WZ, JLH, TYL, and XY analyzed the data. CFG, WZ, TYL, and JLH wrote the manuscript. APZ supervised the project. All authors approved the final version of the submitted manuscript. All authors agreed to be accountable for all aspects of the report.

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Data availability Data are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local laws and regulations of China, where it was conducted. The trial protocol was approved by the Institutional Ethics Committee of Cancer Hospital, Chinese Academy of Medical Sciences (Approval No. 19/208–1992) and registered at ClinicalTrials.gov (NCT04695470).

Consent to participate All patients provided written informed consent prior to screening.

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