FOLFIRI (folinic acid, fluorouracil, and irinotecan) increases not efficacy but toxicity compared with single-agent irinotecan as a second-line treatment in metastatic colorectal cancer patients: a randomized clinical trial

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Abstract

Background: FOLFIRI [irinotecan, folinic acid (CF), and fluorouracil] is considered a standard second-line chemotherapy regimen for patients with metastatic colorectal cancer (mCRC) who failed first-line XELOX/FOLFOX regimens. However, it remains unknown whether fluorouracil is still necessary in this case. This trial was designed to test the superiority of FOLFIRI over single-agent irinotecan as a second-line treatment for patients with mCRC.

Methods: This randomized clinical trial was conducted in five hospitals in China. From 4 November 2016 to 17 January 2020, patients aged 18 years or older with histologically confirmed unresectable mCRC and who had failed first-line XELOX/FOLFOX regimens were screened and enrolled. Patients were randomized to receive either FOLFIRI or irinotecan. The primary endpoint was progression-free survival (PFS). Secondary endpoints included overall survival (OS), objective response rate (ORR), and toxicity. Data were analyzed on an intention-to-treat basis.

Results: A total of 172 patients with mCRC were randomly treated with FOLFIRI ($n = 88$) or irinotecan ($n = 84$). The median PFS was 104 and 112 days (3.5 and 3.7 months) in the FOLFIRI and irinotecan groups, respectively [hazard ratio (HR) = 1.084, 95% confidence interval (CI) = 0.7911–1.485; $p = 0.6094$], and there was also no significant difference in OS and ORR between the two groups. The incidence of the following adverse events (AEs) was significantly higher in the FOLFIRI group than in the irinotecan group: any grade AEs including leucopenia (73.9% *versus* 55.4%), neutropenia (72.7% *versus* 56.6%), thrombocytopenia (31.8% *versus* 18.1%), jaundice (18.2% *versus* 7.2%), mucositis (40.9% *versus* 14.5%), vomiting (37.5% *versus* 21.7%), and fever (19.3% *versus* 7.2%) and grade 3–4 neutropenia (47.7% *versus* 21.7%).

Conclusion: This is the first head-to-head trial showing that single-agent irinotecan yielded PFS, OS, and ORR similar to FOLFIRI, with a more favorable toxicity profile; therefore, it might be a more favorable standard chemotherapy regimen for mCRC patients who failed first-line XELOX/FOLFOX regimens.

Trial registration: This study is registered with ClinicalTrials.gov, number NCT02935764, registered 17 October 2016, https://clinicaltrials.gov/ct2/show/NCT02935764.

Keywords: chemotherapy, FOLFIRI, irinotecan, metastatic colorectal cancer

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Background

Colorectal cancer (CRC) is one of the most common malignant tumors.[1,2] According to recent data, CRC is the third most common cancer worldwide and the second leading cause of cancer death. In China, CRC is the second most common cancer (12.2%).[3–5] Nearly one third of patients have distant metastasis at first diagnosis, and the most common treatment for metastatic colorectal cancer (mCRC) is systemic chemotherapy and molecular targeted drugs.[6]

Fluorouracil (5-FU) and folinic acid (CF) were the most essential therapeutic regimens for patients with mCRC in the first-line setting before 2000. In the 21st century, combination chemotherapies, such as FOLFOX (CF, 5-FU, and oxaliplatin) and FOLFIRI (CF, 5-FU, and irinotecan), began to be utilized, and patients who received all three agents including 5-FU, oxaliplatin, and irinotecan survived longer.[7,8] Currently, the utility of molecular targeted drugs has further improved the efficacy of chemotherapy for mCRC.[9–12]

The V308 study showed that FOLFOX and FOLFIRI regimens could be the first or second line of each other in the treatment of mCRC. Patients with mCRC were randomly assigned to two groups: group A received FOLFIRI as first-line therapy and FOLFOX as second-line treatment; group B received FOLFOX as first-line treatment and FOLFIRI as second-line treatment. The median overall survival (mOS) of groups A and B was 21.5 months *versus* 20.6 months ($p = 0.99$); the median progression-free survival (mPFS) of first-line chemotherapy was 8.5 months *versus* 8.0 months ($p = 0.9$), and the objective response rate (ORR) was 56% *versus* 54% ($p = 0.26$); the mPFS of second-line chemotherapy was 4.2 months *versus* 2.5 months ($p = 0.003$), and ORR was 15% *versus* 4% ($p = 0.05$) in groups A and B. The results indicated that the overall survival (OS) of patients with mCRC who received FOLFOX-FOLFIRI or FOLFIRI-FOLFOX was similar, and FOLFOX or FOLFIRI as the first-line treatment showed similar efficacy. However, the mPFS and ORR of FOLFOX were significantly higher than those of FOLFIRI in the second-line treatment.[13]

Based on the results of similar OS of the two groups in the V308 trial, FOLFOX as first-line treatment followed by FOLFIRI as second-line treatment, or in reverse order, has been considered the standard chemotherapy regimen for unresectable mCRC. In this study, 5-FU was used in both the first- and second-line treatments. However, cancer cells may have developed resistance to 5-FU after first-line 5-FU-containing regimen failure. Therefore, we considered whether it is still necessary to continue to administer 5-FU as a second-line treatment.

In a randomized study, mCRC patients who had failed first-line treatment with irinotecan and intravenous injection of 5-FU/CF (IFL) regimen were randomly divided into three groups: 5-FU/CF, single-agent oxaliplatin, and FOLFOX groups. The results showed that ORR of three groups was 0%, 1.3%, and 9.9%, and mPFS was 2.7, 1.6, and 4.6 months ($p < 0.001$), respectively.[14] This suggested that after the first-line treatment failure with IFL, the efficacy of oxaliplatin in the second-line treatment of mCRC was very limited, and the combination of 5-FU significantly improved the efficacy. Therefore, oxaliplatin needs to be combined with 5-FU as a second-line treatment.

In light of the V308 study, FOLFIRI has been widely recognized as the standard second-line treatment for mCRC after first-line treatment with FOLFOX. Meanwhile, irinotecan monodrug is also a second-line choice recommended by the NCCN guideline after two large phase 3 trials compared irinotecan with either best supportive care (V301 Study) or an infusional 5-FU/CF regimen (V302 Study) as second-line chemotherapy after prior treatment with 5-FU-based regimens and both obtained positive results.[15,16] The FOLFOX and oxaliplatin monodrug comparative study led us to ponder whether FOLFIRI is better than irinotecan monotherapy as a second-line chemotherapy; however, there is no head-to-head comparative study between FOLFIRI and irinotecan. Therefore, we performed this randomized clinical study to investigate whether FOLFIRI is superior to irinotecan as a second-line treatment for patients with mCRC who failed 5-FU-based regimens.

Methods

Study design and participants

This study is a randomized, open-label, multicenter, phase 3 clinical trial performed at five hospitals in China designed to demonstrate the superiority of FOLFIRI over single-agent irinotecan as a second-line treatment of mCRC patients.

Eligible patients were screened and enrolled between 4 November 2016 and 17 January 2020. Eligible patients included aged 18 years or older with histologically confirmed and unresectable colorectal adenocarcinoma and who had withdrawn from the first-line oxaliplatin combined with 5-FU or its derivative chemotherapy (FOLFOX or XELOX) with or without molecular targeted drugs because of disease progression (during chemotherapy or within 3 months after the final dose of chemotherapy), intolerable toxicity, or relapse less than 6 months after the final dose of adjuvant FOLFOX or XELOX chemotherapy. To be eligible, patients also had to have a life expectancy of at least 90 days, an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, at least one measurable disease lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) criteria, and adequate bone marrow, liver, and renal function, and patients voluntarily agreed to participate in this clinical trial. As the molecular targeted drugs bevacizumab and cetuximab are expensive and not included in the Chinese medical insurance in the past, we informed the patients and only enrolled patients whose conditions were not suitable for bevacizumab (contraindications include hemorrhage, obstruction, thrombus within 6 months, uncontrolled hypertension, proteinuria, and unhealed wound) or cetuximab (K-RAS/N-RAS or B-RAF mutation) or were allergic to monoclonal antibodies or unable to afford them economically. Patients were excluded if they had received any anticancer treatment within 14 days before randomization or if irinotecan was used as first-line chemotherapy. Other reasons for exclusion included brain metastases, known deficiency of dihydropyrimidine dehydrogenase, previous chronic inflammatory bowel disease, chronic diarrhea or recurrent bowel obstruction, active severe infection, important organ failure, or other serious diseases.

The protocol was approved by Fudan University Shanghai Cancer Center ethics review board (approval ID: 1608162-17-1612A), and the study was conducted according to the principles of Good Clinical Practice and the Declaration of Helsinki. All patients signed randomization and treatment informed consent form.

Randomization and masking

Eligible patients were randomly assigned (1:1) to either the FOLFIRI or irinotecan treatment group via an interactive web-response system, stratified by ECOG score (0–1 *versus* 2) and number of metastatic sites (1–2 *versus* > 2). As this was an open-label study, the patients, investigators, and study team were not masked to the allocated treatment.

Procedures

Patients in the FOLFIRI group received irinotecan 180 mg/m^2, CF 400 mg/m^2, and fluorouracil 400 mg/m^2 intravenously on day 1, followed by a 46-h continuous infusion of fluorouracil 2400 mg/m^2, repeated every 14 days. Patients in the irinotecan group received irinotecan 180 mg/m^2 every 14 days. We did not define the minimum or maximum number of treatment cycles. Treatment continued until disease progression, intolerable toxicity, or patient refusal. Concurrent anticancer treatments were prohibited. In total, 71 (80.7%) patients in FOLFIRI group and 72 (85.7%) patients in IRI group were tested for UGT1A1 polymorphisms (UGT1A1*28 and UGT1A1*6) at baseline.[17]

We performed computed tomography or magnetic resonance imaging (for only two patients who were allergic to the iodine contrast medium) scans at baseline and every 6 weeks thereafter. Tumor response or progression was evaluated using the RECIST 1.1 criteria. In addition, investigators graded AE (adverse events) using the National Cancer Institute Common Terminology Criteria (NCI-CTCAE version 4.0) and also recorded laboratory parameters of peripheral blood counts and blood biochemistry on the first day of each cycle (or the day before) during the protocol treatment period. Toxicities can be managed by dose reduction or discontinuation of irinotecan or fluorouracil.

Outcomes

The primary endpoint was PFS, defined as the time from the date of randomization to the date of confirmed progression or death from any cause, whichever occurred first. The secondary endpoints included the following: OS (time from the date of randomization to the date of death from any cause), ORR (the proportion of eligible patients with measurable lesions at baseline with the best overall response of complete response or partial response), toxicity and safety, and biomarker analysis. Safety analyses included all patients who received at least one dose of the

study therapy. Biomarker analysis (the correlation between UGT1A1 polymorphisms with effects and toxicity) will be reported elsewhere.

Statistical analysis

This study was designed to assess the superiority of FOLFIRI compared with irinotecan monotherapy in terms of PFS. Considering that the mPFS was 2.6 months for patients treated with irinotecan after failure of oxaliplatin combined with 5-FU in the EPIC study,[18] and the study design was similar to our trial, we selected it to be our assumption for the IRI group mPFS. The mPFS was 2.5–4.7 months for patients treated with FOLFIRI in different trials,[13,19,20] and in reference to the results of the mPFS of patients who received FOLFOX which was 4.6 months, which was superior to that of patients who received single-agent oxaliplatin at 1.6 months in the second-line treatment study,[14] the mPFS was assumed to be 2.5 months for patients treated with irinotecan single agent and 4.0 months for patients treated with FOLFIRI, which had a clinically valuable absolute benefit of 1.5 months longer, with a corresponding hazard ratio (HR) of 0.625. Under these assumptions, a two-sided significance level of 0.05 with 80% power shows the superiority of FOLFIRI *versus* irinotecan, an estimated 148 progression events are needed to show superiority and an enrollment of at least 164 patients (82 in each group) is required, accounting for a 10% dropout rate.

Assessment of treatment efficacy was performed on an intent-to-treat basis. Survival curves were generated using the Kaplan–Meier method, and log-rank tests were used to compare PFS and OS. We used Cox proportional hazards models to calculate HRs with 95% CIs and $P_{interaction}$ values for predefined subgroup analyses. The predefined subgroups included age, sex, ECOG performance status, pathological type, primary tumor location, primary tumor resection, number of metastatic sites, liver metastases, liver-limited metastases, stage at diagnosis (synchronous *versus* metachronous metastasis), RAS status, B-RAF status, and UGT1A1 polymorphism. We performed multivariable analysis using the Cox proportional hazard model, which included the treatment regimen and the above 13 factors. In addition, we assessed between-group differences in the overall response and AEs using χ^2 tests.

All statistical analyses were performed using SPSS (version 26), R project (version 3.6), and Stata (version 16). This study was registered with ClinicalTrials.gov (NCT02935764).

Results

A total of 172 patients were enrolled and randomly assigned to receive either FOLFIRI ($n = 88$) or irinotecan (IRI, $n = 84$) as second-line therapy for mCRC. One patient in the irinotecan group did not receive any treatment (Figure 1). The baseline characteristics were well balanced between the two groups (Table 1).

The median treatment duration was 6 cycles (ranging 1–12 cycles) in the FOLFIRI group and 6 cycles (ranging 0–26 cycles) in the IRI group. Treatment was discontinued in 78 (88.6%) of 88 patients in the FOLFIRI group because of disease progression [58 (65.9%)], AE [9 (10.2%)], patient refusal [7 (8.0%)], and change to other therapy [4 (4.5%)] and 74 (89.2%) of 84 in the IRI group because of disease progression [50 (59.5%)], AE [4 (4.8%)], patient refusal [13 (15.9%)], change to other therapy [6 (7.1%)], and CR [1 (1.2%)]. In addition, the number of patients refused treatment after less than 3 times of treatment in two groups were actually similar (five in FOLFIRI group and seven in IRI group), and the exact reason of refusal was not clear. A delay in the treatment administration was necessary for 42 (47.7%) of 88 patients in the FOLFIRI group and 40 (47.6%) of 84 in the IRI group, and a dose reduction was needed for 17 (19.3%) patients in the FOLFIRI group and 14 (16.7%) in the IRI group, most of which (80%) occurred in patients with the UGT1A1 gene mutation. The relative dose intensity of irinotecan was 85.6% in the FOLFIRI group, and 88.7% in the IRI group.

There was no significant difference in the mPFS between the two groups: 104 days (3.5 months; 95% CI = 85–123 days) in the FOLFIRI group and 112 days (3.7 months; 95% CI = 92–132 days) in the IRI group (HR = 1.084, 95% CI = 0.7911–1.485; $p = 0.6094$) (Figure 2(a)). At the cutoff date for the collection of survival data (14 January 2021), a total of 114 deaths were confirmed (59 in the FOLFIRI group and 55 in the IRI group). With a median follow-up of 388 days [12.9 months; interquartile range (IQR) = 221–521 days] for the entire study population, the mOS was 420 days (14 months; 95% CI = 349–491 days) in the FOLFIRI group and 408 days (13.6 months; 95% CI = 325–491 days) in the IRI group (HR = 0.9466,
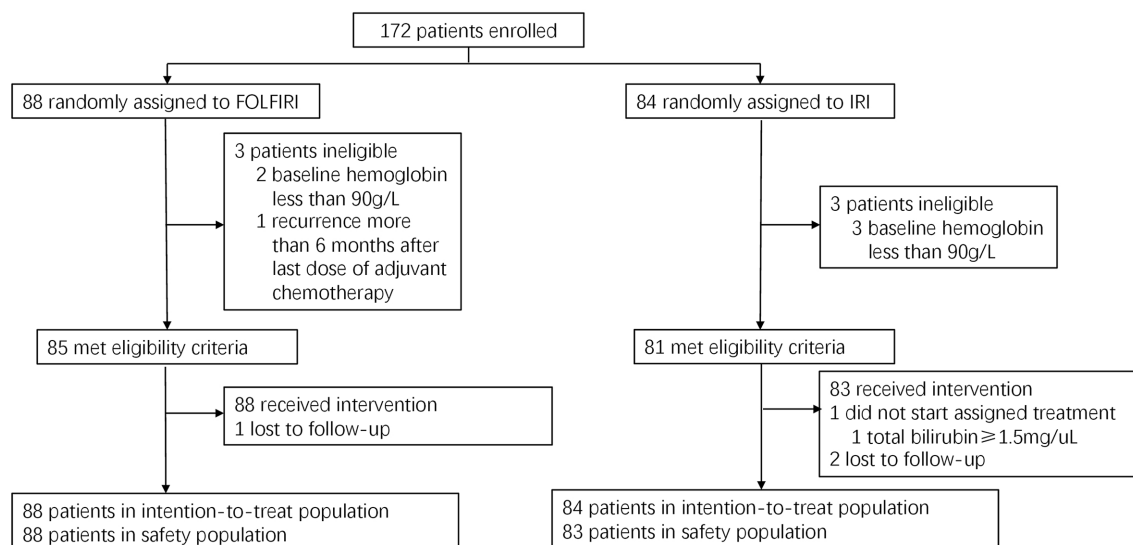
Figure 1. Patient flow diagram.

95% CI = 0.6553–1.368; p = 0.7689) (Figure 2(b)). There were 83 progression or death events in the FOLFIRI group and 80 in the IRI group. Six of the 88 (6.8%, 6 partial remission (PR)) patients in the FOLFIRI group and 6 of 84 (7.1%, 1 complete remission (CR), and 5 PR) in the IRI group had an overall response to treatment (p = 0.933), which was not significantly different. Overall, all the patients in the FOLFIRI and IRI groups had at least one AE during the study (Table 2 listed AE with an incidence rate over 5%). Patients in the FOLFIRI group experienced significantly higher grade AEs, including leucopenia, neutropenia, thrombocytopenia, jaundice, mucositis, vomiting, and fever than those in the IRI group. Patients in the FOLFIRI group had a numerically higher incidence of grade 3–4 AEs than those in the IRI group [47 of 88 (53.4%) *versus* 24 of 83 (28.9%), p = 0.001]. This difference was mainly attributed to grade 3–4 neutropenia, which occurred more frequently in the FOLFIRI group than in the IRI group [42 of 88 (47.7%) *versus* 18 of 83 (21.7%), p < 0.001]. The severity of diarrhea was correlated with UGT1A1 (UGT1A1*6 and UGT1A1*28) polymorphisms in all patients (Spearman p = 0.016) (eFigure 1A). The severity of neutropenia was correlated with UGT1A1 polymorphisms in all patients (Spearman p = 0.073) (eFigure 1B), and the correlation was significant in the IRI group (Spearman p = 0.001) (eFigure 1 C), which may be due to the interference of neutropenia caused by 5-FU in the FOLFIRI group. One patient died 9 days after the last dose of treatment in the

IRI group, which may be related to treatment; however, the cause of death was unknown. No other treatment-related deaths were observed.

According to the exploratory subgroup analysis, FOLFIRI was not superior to IRI in all subgroups in terms of PFS (Figure 3(a)) and OS (Figure 3(b)). In addition, the results of the multivariable analysis using the Cox proportional hazard model, which included a second-line treatment regimen as a factor, revealed that with or without 5-FU, it was not a significant prognostic factor for PFS and OS (eTable1).

Discussion

The effect of the second-line FOLFOX was significantly better than that of single-agent oxaliplatin after the first-line 5-FU-containing regimen failure[14]; however, there is no evidence that second-line FOLFIRI is superior to irinotecan alone. In the FOCUS study, which compared the effects of different strategies of sequential and combination chemotherapy for mCRC, patients were randomly assigned to three groups: group A received 5-FU single-agent until progression and then irinotecan single-agent as second-line treatment; group B received 5-FU single-agent until progression and was then divided into two subgroups that received different combined chemotherapy (irinotecan plus 5-FU or oxaliplatin plus 5-FU); group C received first-line combined chemotherapy. The results showed that in the second-line treatment, the ORR of single-agent irinotecan in

Table 1. Baseline characteristics.

	No.(%)	
	FOLFIRI (*n* = 88)	IRI (*n* = 84)
Age (years)		
<65	59 (67)	52 (62)
⩾65	29 (33)	32 (38)
Median (IQR)	59 (50–66)	61 (53–68)
Sex		
Men	52 (59)	54 (64)
Women	36 (41)	30 (36)
ECOG performance status[a]		
0–1	86 (98)	83 (99)
2	2 (2)	1 (1)
Stage at diagnosis		
Synchronous metastasis	56 (64)	54 (64)
Metachronous metastasis	32 (36)	30 (36)
Primary tumor location		
Right side	27 (31)	21 (25)
Left side	61 (69)	63 (75)
Primary tumor resection		
Yes	64 (73)	54 (64)
No	24 (27)	30 (36)
Pathological type		
Adenocarcinoma	73 (83)	73 (87)
Mucinous adenocarcinoma/ signet ring	15 (17)	11 (13)
Number of metastatic sites		
1–2	50 (57)	46 (55)
>2	38 (43)	38 (45)
Liver metastases		
Yes	57 (65)	56 (67)
No	31 (35)	28 (33)

(Continued)

Table 1. (Continued)

	No.(%)	
	FOLFIRI (*n* = 88)	IRI (*n* = 84)
Liver-limited metastases		
Yes	21 (24)	20 (24)
No	67 (76)	64 (76)
***UGT1A1* polymorphism**		
Wild	37 (42)	36 (43)
Single heterozygote	26 (30)	31 (37)
Double heterozygotes or homozygotes	8 (9)	5 (6)
Unknown	17 (19)	12 (14)
***K-RAS* and *N-RAS* status**		
Wild	43 (49)	32 (38)
Mutant	36 (41)	34 (41)
Unknown	9 (10)	18 (21)
***BRAF* status**		
Wild	75 (85)	63 (75)
Mutant	4 (5)	3 (4)
Unknown	9 (10)	18 (21)

ECOG, Eastern Cooperative Oncology Group; FOLFIRI, folinic acid, fluorouracil, and irinotecan; IRI, irinotecan. Data are *n* (%) or median (IQR).
[a]Assessed according to ECOG guidelines.

group A and irinotecan combined with 5-FU in the subgroup of group B was 11.0% and 16.0%, respectively ($p = 0.07$), and the mPFS was 4.3 and 4.4 months, respectively ($p = 0.75$). Although the ORR of irinotecan combined with 5-FU was slightly higher than that of irinotecan alone, the PFS was similar.[21]

However, there was no head-to-head trial to compare the effects of irinotecan combined with 5-FU regimen with single-agent irinotecan, and FOLFIRI with or without target drugs is considered the standard second-line treatment. Therefore, we conducted this study, assuming that the FOLFIRI regimen is superior to irinotecan monotherapy. However, the results showed that FOLFIRI was not superior to irinotecan in
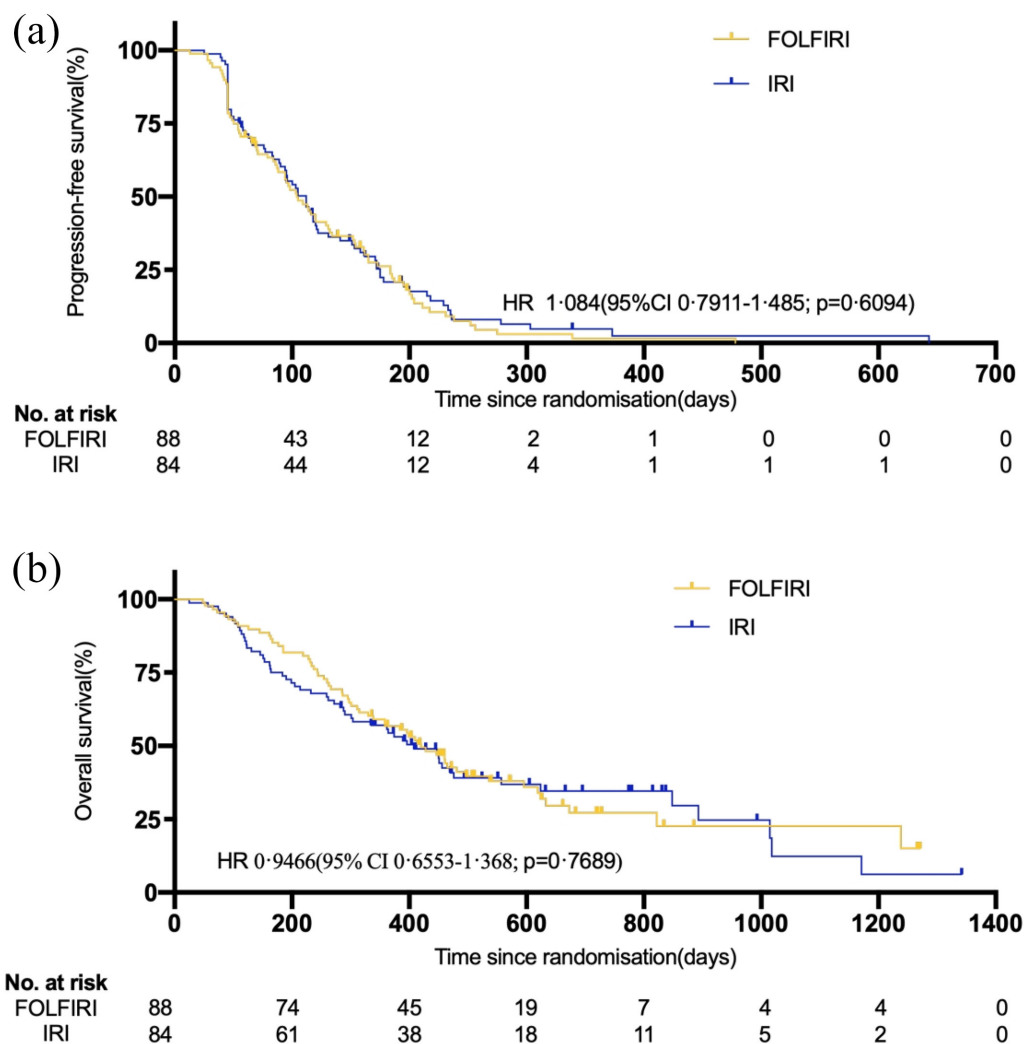
Figure 2. Kaplan–Meier survival estimates in the intention-to-treat population. (a) Progression-free survival and (b) overall survival in patients receiving FOLFIRI compared with patients receiving IRI.

terms of PFS in all patients and every subgroup, and no significant differences were detected in OS and ORR between the two groups. Importantly, the PFS, OS, and ORR data between the two groups were very close, with HRs for PFS and OS close to 1. Furthermore, the multivariable analysis using the Cox proportional hazard model, which included the treatment regimen as a factor, showed that with or without 5-FU, it was not a significant *independent factor associated with PFS or OS*, which further demonstrated that adding 5-FU did not improve treatment efficacy and prognosis. Meanwhile, FOLFIRI resulted to more AEs. Patients in the FOLFIRI group had significantly higher grade AEs, including leucopenia, neutropenia, thrombocytopenia, jaundice, mucositis, and vomiting and had a numerically higher incidence of grade 3–4 AEs, especially neutropenia, compared with patients in the irinotecan group. Treatment discontinuation due to disease progression was similar in both groups; however, treatment discontinuations (10.2% *versus* 4.8%) were higher in the FOLFIRI group than in the irinotecan group. These results suggested that adding 5-FU to irinotecan after the first-line 5-FU-containing chemotherapy failure did not enhance the effects but rather increased the toxicity as the cancer cells already became resistant to 5-FU, and continuation of 5-FU in this case is no longer warranted. Most patients were enrolled because of progression after first-line treatment, only a few patients were enrolled because of intolerable toxicity in first-line treatment. Specifically, in FOLFIRI group, six patients

**Table 2.** Adverse events (safety population).

	Any grade			Grade ¾		
	IRI (*N* = 83)	FOLFIRI (*N* = 88)	*p* value	IRI (*N* = 83)	FOLFIRI (*N* = 88)	*p* value
Hematological						
Leucopenia	46 (55)	65 (74)	0.012	14 (17)	22 (25)	0.192
Neutropenia	47 (57)	64 (73)	0.027	18 (22)	42 (48)	0.000
Thrombocytopenia	15 (18)	28 (32)	0.038	4 (5)	2 (2)	0.367
Anemia	26 (31)	35 (40)	0.249	0 (0)	2 (2)	0.173
Non-hematological						
ALP abnormality	24 (29)	33 (38)	0.234	0 (0)	0 (0)	
ALT abnormality	7 (8)	15 (17)	0.093	0 (0)	0 (0)	
AST abnormality	8 (10)	16 (18)	0.108	0 (0)	0 (0)	
Jaundice	6 (7)	16 (18)	0.033	0 (0)	0 (0)	
Diarrhea	29 (35)	39 (44)	0.210	4 (5)	5 (6)	0.801
Mucositis	12 (14)	36 (41)	0.000	0 (0)	0 (0)	
Anorexia	31 (37)	39 (44)	0.354	1 (1)	0 (0)	0.303
Nausea	33 (40)	42 (48)	0.294	0 (0)	0 (0)	
Vomiting	18 (22)	33 (38)	0.024	1 (1)	1 (1)	0.967
Alopecia	46 (55)	52 (59)	0.628	0 (0)	0 (0)	
Fatigue	39 (47)	49 (56)	0.256	1 (1)	0 (0)	0.303
Weight loss	10 (12)	12 (14)	0.669	0 (0)	0 (0)	
Peripheral sensory neuropathy	8 (10)	9 (10)	0.898	0 (0)	0 (0)	
Fever	6 (7)	17 (19)	0.021	0 (0)	0 (0)	
Febrile neutropenia	3 (4)	8 (9)	0.145	3 (4)	8 (9)	0.145

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FOLFIRI, folinic acid, fluorouracil, and irinotecan; IRI, irinotecan. Data are *n* (%).

were intolerable for oxaliplatin and two patients were intolerable for 5-FU; in IRI group, nine patients were intolerable for oxaliplatin and one patient was intolerable for 5-FU. Therefore, most patients have had the opportunity to develop resistance. The results could also answer the question why the effects of FOLFOX and FOLFIRI were similar in terms of ORR and PFS in the first-line treatment, but the effect of FOLFOX was better than that of FOLFIRI in the second-line treatment in the V308 study, as the application of 5-FU in the second-line treatment increased the effects of oxaliplatin but not irinotecan. The synergistic effect between oxaliplatin and 5-FU has been demonstrated in both *in vitro* and *in vivo* experiments[22] and clinical trials.[23–25] Moreover, the addition of oxaliplatin could overcome resistance to 5-FU,[26] which could explain why the addition of 5-FU after development of drug resistance in the second-line oxaliplatin-based regimens could still improve the effects. However, the combination of irinotecan and 5-FU in previous studies was either antagonistic or synergistic,[27–29] and irinotecan has not been reported to overcome resistance to 5-FU. Our study showed that the combination of 5-FU
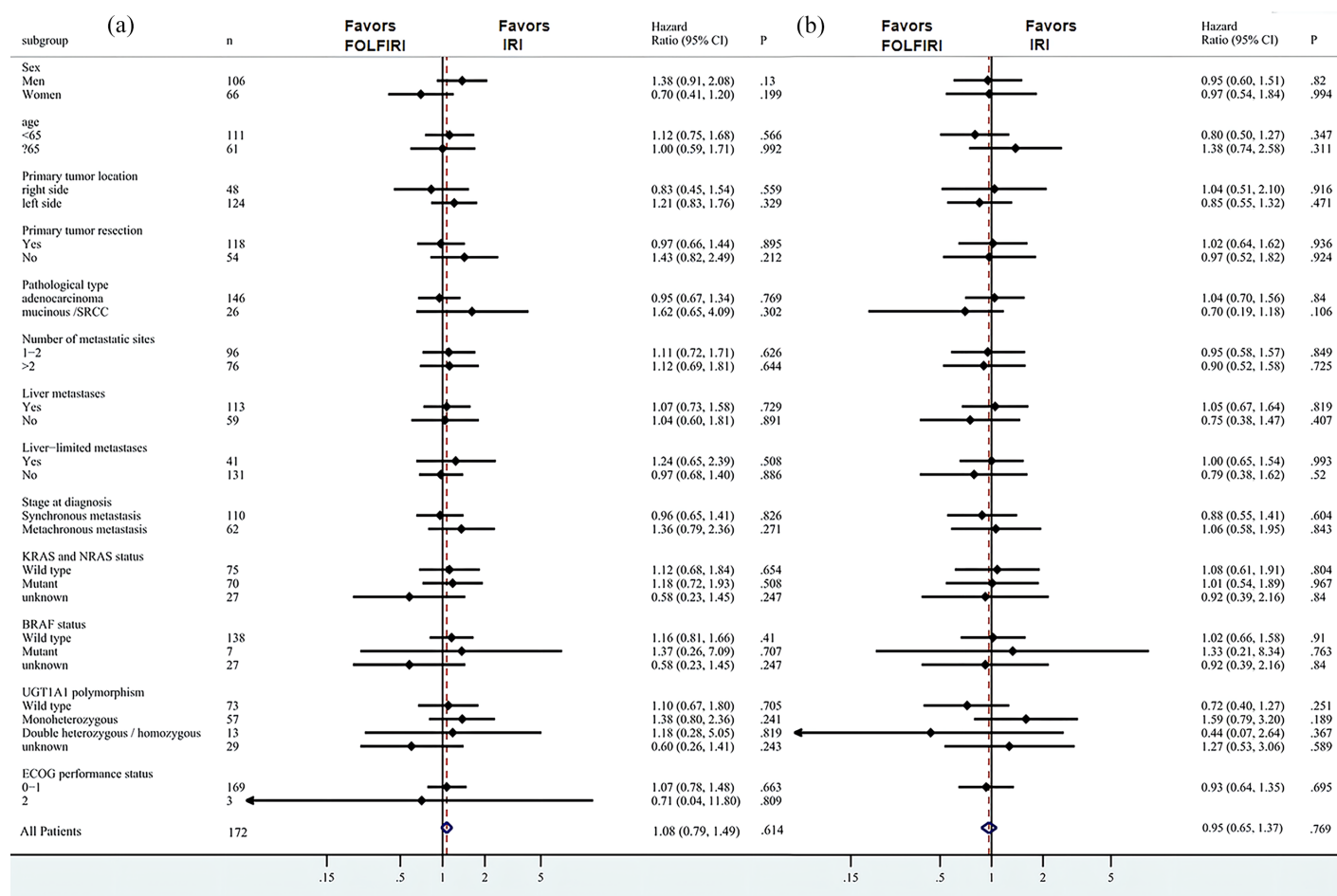
Figure 3. Forest plots of exploratory subgroup analysis of (a) disease-free survival and (b) overall survival.

after development of drug resistance in the second-line irinotecan-based regimens did not improve the effects, which indicated that irinotecan might not overcome resistance to 5-FU.

To our knowledge, our study is the first head-to-head trial showing that FOLFIRI failed to improve PFS, OS, and ORR compared with irinotecan as second-line treatment for mCRC, and single-agent irinotecan demonstrated significantly less toxicity than FOLFIRI. Therefore, irinotecan as a single-agent might be a more favorable chemotherapy option and might be a choice for second-line treatment, as chemotherapy combined with target drugs is currently the standard of care for mCRC patients.

Oral 5-FU prodrug S-1 is widely used as a substitute for 5-FU continuous infusion. The FIRIS study demonstrated the noninferiority of irinotecan plus S-1 to FOLFIRI in terms of PFS and OS

as second-line chemotherapy for mCRC,[30,31] suggesting that the IRIS regimen could be an option as a second-line chemotherapy for mCRC. Subsequently, IRIS-based treatment (combined with target drugs) has been further investigated as a second-line treatment for mCRC.[32,33] Another oral 5-FU prodrug capecitabine was also widely used. When combined with oxaliplatin as first-line treatment for mCRC, capecitabine can be used as a substitute for 5-FU continuous infusion, as the effects of oxaliplatin plus capecitabine (XELOX) were similar to FOLFOX.[34,35] However, irinotecan combined with capecitabine (XELIRI) led to a significantly greater gastrointestinal toxicity and inferior PFS compared with FOLFIRI in the first-line setting in the BICC-C trial.[36] The AXEPT study showed that the modified XELIRI (mXELIRI) with or without bevacizumab was well tolerated and was noninferior to FOLFIRI with or without bevacizumab in terms of OS, suggesting that mXELIRI could be an

alternative to FOLFIRI as a second-line treatment for mCRC, at least in Asian patients.[9] In FIRIS and AXEPT studies, the value of the continued use of 5-FU in the FOLFIRI regimen was not considered. The results of our study suggest that it is not necessary to continue 5-FU in the second-line irinotecan-based treatment after failure of the first-line 5-FU-based regimen, which only increased the toxicity but not the efficacy. S-1 or capecitabine might also be unnecessary in this setting, as they were transformed to 5-FU *in vivo* to exert anticancer effects, and the effects of IRIS or mXELIRI were similar to those of FOLFIRI. Therefore, single-agent irinotecan could be the best choice as a second-line chemotherapy for mCRC after failure of the first-line 5-FU-based treatment, as data showed its almost similar effects but with a significantly less toxicity compared with FOLFIRI in our study. Moreover, single-agent irinotecan could eliminate the inconvenience of a continuous infusion of 5-FU and implantation of an intravenous port system in the FOLFIRI regimen, as well as the side effects and expenses caused by S-1 or capecitabine in the IRIS or mXELIRI regimen.

Mutations in the drug metabolism enzyme UGT1A1 are associated with the severity of major AEs caused by irinotecan, including diarrhea and neutropenia, which indicates the value of UGT1A1 for toxicity prediction and is expected to guide clinical personalized medicine and dosage modification in the future.

Our study has some limitations. First, quality-of-life assessments are crucial to patients receiving palliative treatments, and relevant data might be useful in the treatment decision-making process regarding irinotecan monodrug or FOLFIRI regimens. Second, the trial was open-label, which might introduce a potential reporting bias. Third, the lack of standard monoclonals in first-line therapy may be a limitation to the external validity of this trial.

Conclusion
Our study showed that FOLFIRI failed to improve the effects compared with single-agent irinotecan as a second-line treatment for mCRC. However, single-agent irinotecan was significantly less toxic than FOLFIRI. The results suggested that single-agent irinotecan might be a more favorable second-line chemotherapy, with similar efficacy and less toxicity, and offers more convenience compared with FOLFIRI for patients with mCRC.

Ethics approval and consent to participate
The protocol was approved by Fudan University Shanghai Cancer Center ethics review board (approval ID: 1608162-17-1612A), and the study conduct was conducted according to the principles of Good Clinical Practice and the Declaration of Helsinki. All patients had signed randomization and treatment informed consent form.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article and its supplementary information files.

Supplemental material
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