Peer Review File

Article information: https://dx.doi.org/10.21037/jgo-24-76

Reviewer A

General Review/Comments

This is a retrospective study that evaluates the use of chemotherapy in heavily pre-treated patients with metastatic colorectal cancer. The study examined 13 patients who received gemcitabine plus raltitrexed or S1.

It would be beneficial to know the specific AE's, dose reductions, or any changes made to the patients receiving CT.

Specific Manuscript Comments:

Comment 1: Recommend checking English grammar as several sentences are difficult to understand.

Reply 1: We have revised the grammar of some sentences.

Comment 2: Page 4 line 76-83: please summarize it in a simpler, more clear text.

Reply 2: We have revised the expression of this paragraph.

Changes in the text:

S-1 is an oral fluoropyrimidine derivative composed of tegafur (FT), gimeracil (CDHP), and oteracil potassium (Oxo). After oral administration, FT is gradually converted into 5-fluorouracil (5-FU). CDHP enhances the concentration of 5-FU by reversibly inhibiting DPD, which is the catabolic enzyme of fluorouracil present in the liver. Oxo can selectively and reversibly inhibit the activity of the 5-FU distributed in the gastrointestinal tract and thus decreases gastrointestinal toxicity without affecting the antitumor activity of 5-FU (15).↵

Comment 3: Page 5 line 111: "third- or later-line treatment" The title implies comparison to third-line therapy. However, the research says it has been used in three or more advanced phases. Modify the title and separate third- and fourth-line survival data in the text.

Reply 3: Currently, TAS-102, regorafenib, and fruquintinib are the recommended third-line regimens for metastatic colorectal cancer. There is no consensus on the fourth- and later-line treatment of mCRC. In clinical work, the selection of regimens is often affected by the patient's physical condition, economic condition, personal willingness, adverse effects and other factors. It is usually difficult to make ideal regimens fully according to the disease guidelines, which leads to standard third-line drugs being used in the subsequent treatments. Meanwhile, the treatment of gemcitabine plus raltitrexed or S-1 showed the similar efficacy of the chemotherapy regimen to the other three drugs which increased our confidence in the prior use of the chemotherapy regimen. After discussion, we still retained the expression of the "third-line treatment" in the title.

Because of the small number of included cases and the large difference between the number of third-line and later-line cases, there may exist a large bias. The relevant data were only mentioned in the clinical characteristics, and no further analysis of survival data was carried out.

Changes in the text: None.

Comment 4: Page 6 line 167-168: The OS should be the third line's treatment date, followed by the fourth line's treatment date, for each line individually.

Reply 4: We have redefined OS as "from the initiation of the target regimen application to death or the last follow-up date if the patient was still alive". The median OS of the chemotherapy, fruquintinib, regorafenib, and TAS-102 groups was 7.4, 6.1, 8.3, and 6.7 months (P=0.384), respectively (Figure 2-Revised).

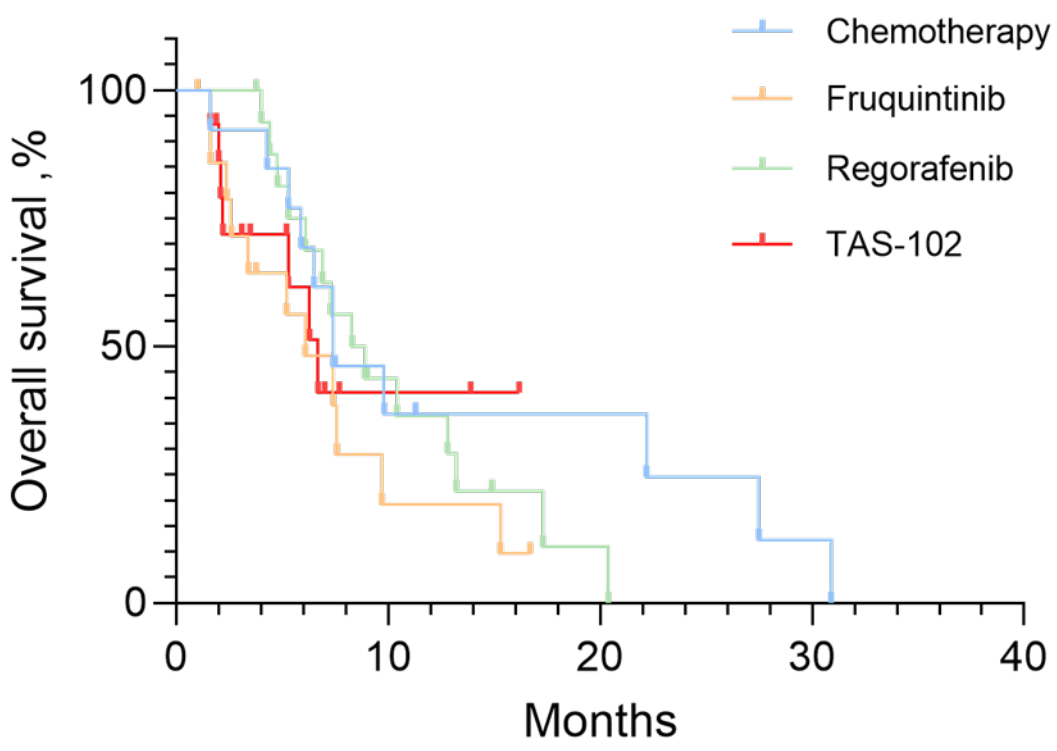

Figure 2-revised Kaplan-Meier analysis of overall survival.

Comment 5: Page 8 line 231: The research should concentrate on all patients who have undergone CT, as opposed to a single case report. Alternatively, case studies could be incorporated into the redesign of the research.

Reply 5: Due to the excellent therapeutic effect of this case, we present this case in this study to support the feasibility of this chemotherapy regimen.

Changes in the text: None.

Comment 6: Page 9 line 237:' treated with FOLFRI and Xeloda for 13 months' please check the regimen.

Reply 6: We are sorry for the ambiguity caused by our inappropriate expression. We have revised the statement.

Changes in the text:

A 64-year-old man was diagnosed with right colon cancer with synchronous liver metastases. The patient underwent radical right hemicolectomy and postoperative adjuvant chemotherapy with FOLFOX. Four months after his surgery, the patient developed a new metastasis in the lesser curvature of the stomach He received FOLFIRI as the first-line treatment. XELOX was replaced as the second-line treatment when the lung metastasis occurred during follow-up. During this period, the patient underwent three CT-guided radiofrequency ablations (RFA). First- and second-line treatments lasted for a total of 13 months. After second-line treatment, disease progression recurred

Comment 7: The organization of the discussion can be reworked. Please provide separate clarification for patients who have undergone chemotherapy regimens.

Reply 7: We add the separate clarification for patients who have undergone chemotherapy regimens (Table 2).

Changes in the text:

Table 2 Patient characteristics of chemotherapy group

Characteristics	Gemcitabine plus raltitrexed (N=8)	Gemcitabine plus S-1 (N=5)
Sex		
Male	4(30.8)	4(30.8)
Female	4(30.8)	1(7.7)
Age, years	50[38-57]	62.5[50-76]
≤65	5(38.5)	5(38.5)
>65	3(23.1)	0
Primary location		
Colon	4(30.8)	3(23.1)
Rectum	4(30.8)	2(15.4)
Primary tumor resection		
Yes	7(54.8)	5(38.5)
No	1(7.7)	0
Time to metastasis		
Synchronous	3(23.1)	4(30.8)
Metachronous	5(38.5)	1(7.7)

Metastasis management		
Yes	4(30.8)	4(30.8)
No	4(30.8)	1(7.7)
Number of metastatic organs		
<3	8(61.5)	1(7.7)
≥3	0	4(30.8)
Gene mutation status		
Wild type	6(46.2)	5(38.5)
Mutant	2(15.4)	0
Line of treatment		
≤3	3(23.1)	1(7.7)
>3	5(38.5)	4(30.8)

Data are presented as median [range] or number (percentage).

Reviewer B

Comment 1: First of all, my major concern for this study is the very small sample size, which cannot answer the research questions of this study. The authors need to reconsider whether the current study of the 13 patients of interest should be re-written as a case report.

Reply 1: As mentioned in the discussion, limited by the hospital scale and the patient number, we have made our best efforts to enroll eligible patients in this study. The preliminary result revealed that the efficacy and safety of gemcitabine plus raltitrexed or S-1 were similar to the current standard third-line regimens, which rase our confidence to continue to try this regimen further. In the future, we will try to expand the enrollment by conducting multicenter studies with various hospitals.

Changes in the text: None.

Comment 2: Second, the abstract needs some revisions. The background did not explain why gemcitabine plus raltitrexed or S-1 is effective and safe and what the

current knowledge gap is. The results need to briefly summarize the clinical characteristics of the four groups and their baseline comparability. The current conclusion should be tone down since the findings did not support this statement.

Reply 2: We added the relevant literature which demonstrated the efficacy and tolerable toxicity of gemcitabine plus raltitrexed or S-1 in the abstract. With the employment of chi-square test, we found that there were differences of baseline comparisons in metastasis management, number of metastatic organs and gene mutation status among the four groups. We revised the conclusion according the comment.

Changes in the text:

The combination of gemcitabine with raltitrexed or S-1 has been proven effective as a therapy for pancreatic cancer (16-18) and biliary tract cancer (19) with tolerable toxicity. Raltitrexed was demonstrated to have a similar effect like 5-FU (20) while being more suitable for patients with mCRC and cardiologic risk factors or previous cardiotoxicity. Thus, in this study, we selected gemcitabine plus raltitrexed as the preferred regimen. Small-scale research has suggested the effectiveness of S-1 as a third- or later-line regimen for patients with refractory mCRC (21-23). Furthermore, S-1 has been proven to be highly effective in gastric cancer and pancreatic cancer with peritoneal metastasis by virtue of its high rate of transition into the peritoneal cavity (24,25). On the basis of the results of previous studies (24,25), we selected gemcitabine plus S-1 to treat patient

with mCRC and peritoneal metastasis.

Table 1 Patient characteristics

Characteristics	All (N=60)	Chemotherapy (N=13)	Fruquintinib (N=15)	Regorafenib (N=17)	TAS-102 (N=15)	P value
Sex						0.724
Male	38 (63.3)	8 (13.3)	11 (18.3)	11 (18.3)	8 (13.3)	
Female	22 (36.7)	5 (8.3)	4 (6.7)	6 (10.0)	7 (11.7)	
Age, years						0.942
Median[range]	60.6 [30–82]	57.7 [38–76]	58.1 [30–77]	63.8 [47–82]	61.9 [47–78]	
≤65	43 (71.7)	10 (16.7)	11 (18.3)	12 (20.0)	10 (16.7)	
>65	17 (28.3)	3 (5.0)	4 (6.7)	5 (8.3)	5 (8.3)	
Primary location						0.572
Colon	39 (65.0)	8 (13.3)	12 (20.0)	10 (16.7)	9 (15.0)	
Rectum	21 (35.0)	5 (8.3)	3 (5.0)	7 (11.7)	6 (10.0)	
Primary tumor resection						0.918
Yes	54 (90.0)	12 (20.0)	14 (23.3)	15 (25.0)	13 (21.7)	
No	6 (10.0)	1 (1.7)	1 (1.7)	2 (3.3)	2 (3.3)	
Time to metastasis						0.513
Synchronous	33 (55.0)	7 (11.7)	9 (15.0)	7 (11.7)	10 (16.7)	
Metachronous	27 (45.0)	6 (10.0)	6 (10.0)	10 (16.7)	5 (8.3)	
Metastasis management						0.001
Yes	38 (63.3)	8 (13.3)	10 (16.7)	10 (16.7)	10 (16.7)	
No	22 (36.7)	5 (8.3)	5 (8.3)	7 (11.7)	5 (8.3)	
Number of metastatic organs						0.050
<3	9 (15.0)	1 (1.7)	5 (8.3)	0	3 (5.0)	
⩾3	51 (85.0)	12 (20.0)	10 (16.7)	17 (28.3)	12 (20.0)	
Gene mutation status						0.005
Wild type	30 (50.0)	11 (18.3)	3 (5.0)	10 (16.7)	6 (10.0)	
Mutant	30 (50.0)	2 (3.3)	12 (20.0)	7 (11.7)	9 (15.0)	
Line of treatment						0.514
⩽3	17 (28.3)	4 (6.7)	2 (3.3)	6 (10)	5 (8.3)	
>3	43 (71.7)	9 (15.0)	13 (21.7)	11 (18.3)	10 (16.7)	

Data are presented as median [range] or number (percentage).

#Conclusions

In summary, the gemcitabine plus raltitrexed or S-1 regimen achieved a therapeutic effect not worse off than that in the currently practiced standard third-line treatments. With certain therapeutic effect, tolerable adverse reactions and low cost, this regimen represents a potentially therapeutic option for patients with mCRC in clinical work.

Comment 3: Third, the introduction of the main text needs to explain why the authors did not conduct a RCT to examine the efficacy of gemcitabine plus raltitrexed or S-1 and analyze its potential safety for mCRC.

Reply 3: A randomized controlled trial (RCT) needs to meet stringent conditions including randomization, adequate sample size, unbiased outcomes and blinding and so on. The above conditions could not be met due to the limitations of the hospital

size, numbers of patients and participating researchers. In addition, every treatment opportunity for mCRC patients is precious. It is not appropriate to conduct an RCT before the preliminary evaluation of efficacy and tolerable toxicity. Thus, we conducted this retrospective study to assess the feasibility of a future RCT.

Changes in the text:

#Introduction

A randomized controlled trial (RCT) needs to meet stringent conditions including randomization, adequate sample size, unbiased outcomes and blinding and so on. The above conditions could not be met due to the limitations of the hospital size, numbers of patients and participating researchers. In addition, every treatment opportunity for mCRC patients is precious. It is not appropriate to conduct an RCT before the preliminary evaluation of efficacy and tolerable toxicity. Thus, we conducted this retrospective study to assess the feasibility of a future RCT.↵

##Study design and patient population↵

↵

We enrolled patients with mCRC who received gemcitabine plus raltitrexed or S-1, TAS-102, regorafenib, or fruquintinib at the Second Affiliated Hospital of Soochow University from April 1, 2018 to October 31, 2022. Limited by the size of the hospital and the number of patients who underwent treatments, we failed to enroll the expected number of cases. Anyway, we included as many patients as possible who met the inclusion criteria in the study. Data on the following clinical characteristics were collected from these patients: sex, age, primary location, primary tumor resection, time to metastasis, metastasis management, number of metastatic organs, gene mutation status, and line of treatment.↵

Comment 4: Fourth, in the methodology, please describe the details of sample size estimation procedures and details of follow up. In statistics, please describe the test of baseline comparability across the four groups and P value for statistical significance.

Reply 4: Limited by the size of the hospital and the number of patients who underwent treatments, we failed to enroll the expected number of cases. Anyway, we included as many cases as possible that met the inclusion criteria. In the part of "Efficacy and safety assessment", we have made a simple statement of the follow-up methods. According to the comment, we added some details. The chi-square test was applied to compare the constituent ratio among the four groups. The corresponding P values have been added to Table 1.

##Study design and patient population

We enrolled patients with mCRC who received gemcitabine plus raltitrexed or S-1, TAS-102, regorafenib, or fruquintinib at the Second Affiliated Hospital of Soochow University from April 1, 2018 to October 31, 2022. Limited by the size of the hospital and the number of patients who underwent treatments, we failed to enroll the expected number of cases. Anyway, we included as many patients as possible who met the inclusion criteria in the study. Data on the following clinical characteristics were collected from these patients: sex, age, primary location, primary tumor resection, time to metastasis, metastasis management, number of metastatic organs, gene mutation status, and line of treatment.

##Efficacy and safety assessment

During treatment, clinical and imaging follow-up with contrast-enhanced computerized tomography (CT) and enhanced magnetic resonance imaging (MRI) were performed every 3 months. Laboratory tests, including blood routine, biochemistry, and serum tumor markers detection, were performed every 3 weeks. The patients with disease progression were followed up by telephone every 1 month until death or the last follow-up date if the patient was still alive. The tumor response was assessed according to

##Statistical analysis

The study endpoints included progression-free survival (PFS) and overall survival (OS). The PFS was estimated from the initiation of the regimen to disease progression or death without evidence of progression. The OS was recorded from the initiation of the target regimen application to death or the last follow-up date if the patient was still alive. The chi-square test was applied to compare the constituent ratio among the groups. Survival analysis was performed using GraphPad Prism 8.4.2 (GraphPad Software Inc., La Jolla, CA, USA) with the Kaplan-Meier method for median estimation and the 95% confidence interval (CI) for the incidence of events. The log-rank test was used for subgroup analysis. Cox regression analysis was used to investigate potential predictors of survival. Statistical analysis was conducted with SPSS software 25.0 (IBM Corp., Armonk, NY, USA).

Table 1 Patient characteristics

Characteristics	All (N=60)	Chemotherapy (N=13)	Fruquintinib (N=15)	Regorafenib (N=17)	TAS-102 (N=15)	P value
Sex						0.724
Male	38 (63.3)	8 (13.3)	11 (18.3)	11 (18.3)	8 (13.3)	
Female	22 (36.7)	5 (8.3)	4 (6.7)	6 (10.0)	7 (11.7)	
Age, years						0.942
Median[range]	60.6 [30–82]	57.7 [38–76]	58.1 [30–77]	63.8 [47–82]	61.9 [47–78]	
⩽65	43 (71.7)	10 (16.7)	11 (18.3)	12 (20.0)	10 (16.7)	
>65	17 (28.3)	3 (5.0)	4 (6.7)	5 (8.3)	5 (8.3)	
Primary location						0.572
Colon	39 (65.0)	8 (13.3)	12 (20.0)	10 (16.7)	9 (15.0)	
Rectum	21 (35.0)	5 (8.3)	3 (5.0)	7 (11.7)	6 (10.0)	
Primary tumor resection						0.918
Yes	54 (90.0)	12 (20.0)	14 (23.3)	15 (25.0)	13 (21.7)	
No	6 (10.0)	1 (1.7)	1 (1.7)	2 (3.3)	2 (3.3)	
Time to metastasis						0.513
Synchronous	33 (55.0)	7 (11.7)	9 (15.0)	7 (11.7)	10 (16.7)	
Metachronous	27 (45.0)	6 (10.0)	6 (10.0)	10 (16.7)	5 (8.3)	
Metastasis management						0.001
Yes	38 (63.3)	8 (13.3)	10 (16.7)	10 (16.7)	10 (16.7)	
No	22 (36.7)	5 (8.3)	5 (8.3)	7 (11.7)	5 (8.3)	
Number of metastatic organs						0.050
<3	9 (15.0)	1 (1.7)	5 (8.3)	0	3 (5.0)	
⩾3	51 (85.0)	12 (20.0)	10 (16.7)	17 (28.3)	12 (20.0)	
Gene mutation status						0.005
Wild type	30 (50.0)	11 (18.3)	3 (5.0)	10 (16.7)	6 (10.0)	
Mutant	30 (50.0)	2 (3.3)	12 (20.0)	7 (11.7)	9 (15.0)	
Line of treatment						0.514
⩽3	17 (28.3)	4 (6.7)	2 (3.3)	6 (10)	5 (8.3)	
>3	43 (71.7)	9 (15.0)	13 (21.7)	11 (18.3)	10 (16.7)	

Data are presented as median [range] or number (percentage).

Comment 5: Finally, please consider to cite several related papers: 1. Hua S, Gao J, Xu Q, Hong X, Wu W. Pathological complete response in a patient with locally advanced pancreatic adenocarcinoma treated with neoadjuvant gemcitabine and S-1: a case report and literature review. Gland Surg 2022;11(2):494-503. doi: 10.21037/gs-22-6. 2. Wan Y, Luo D. Using a combination of fruquintinib, raltitrexed, and S-1 as a third-line treatment for metastatic colorectal cancer with co-existence of Hodgkin lymphoma: a case report. J Gastrointest Oncol 2023;14(1):450-457. doi: 10.21037/jgo-23-39. 3. Dai Y, Sun L, Zhuang L, Zhang M, Zou Y, Yuan X, Qiu H. Efficacy and safety of low-dose apatinib plus S-1 versus regorafenib and fruquintinib for refractory metastatic colorectal cancer: a retrospective cohort study. J Gastrointest Oncol 2022;13(2):722-731. doi: 10.21037/jgo-22-285.

Reply 5: We have cited the related papers in our article.