Peer Review File

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Reviewer A

This manuscript reports a single arm, phase II study of toripalimab and fruqunitinib in patients with advanced colorectal cancer.

Major comments:

1. There is no statistical justification for sample size.

Reply : As for the calculation process of sample size, we have described it in detail in the part of "Study design and participants" (marked with red color), please review it again.

Changes in the text: Changes was marked with red color in the lines of 233-239 of the revised manuscript.

2. The patient population might not be refractory to current available therapies. For example, 16/19 patients had analysis for RAS mutation, 8 patients had RAS mutation, only 6 patients received cetuximab. Therefore, it is difficult to compare results from this study with those from other studies. This limitation should be acknowledged.

Reply : We have to admit that the sample size of the study was small, only 6 patients with RAS wild-type received cetuximab, other 2 patients didn't receive cetuximab because of allergies to it. So now we are conducting a study on large samples, hoping to get better results.

Changes in the text: Without any change in the revised manuscript.

3. Table 3 and Table 4 do not add much to the paper. Findings from these analysis are not reliable, given the small sample size. They should be deleted.

Reply : According to your comment. We have revised the manuscript and deleted the Table 3 and Table 4 in the revised manuscript.

Changes in the text: We have deleted Table 3 and Table 4. Meanwhile we have replaced Table 5 with Table 3 in the revised manuscript.

4. Results showing in Figure 1A are not consistent with those in other tables/figures. For example, 9 patients were shown to have > 20% increase in tumor size as best response (by definition PD) in Figure 1A. In Figure 1B, only 4 patients were labelled as PD.

Reply : Thank you for pointing this out. We have checked Figure 1A and made some corrections in the revised the manuscript.

Changes in the text: We have deleted the Figure1 and reinserted the newly made Figure 1.

5. Other important and relevant genomic alterations, such as BRAF mutations, were not reported.

Reply : Thank you for your suggestion. We re-examined the genetic test results of the enrolled patients and found that the BRAF gene status was the wild type in all 19 patients enrolled.
Changes in the text: We added the results of BRAF gene test in Table 1（marked with red color）.
6. There are instances where authors' intention was not clear. For example, line 357 " the enrolled patients were not thoroughly screened"; line 314, " patients enrolled were usually very complex.." line 191, "13 patients had a history of liver metastasis" (a history of liver metastasis implies that these patients underwent liver resection for liver metastasis).
Reply : Thank you for pointing this out. We have revised the sentence " the enrolled patients were not thoroughly screened" (lines in 458-459) and we deleted the sentence " patients enrolled were usually very complex.." because it was misexpressed (lines in 415-416). For 13 patients had a history of liver metastasis, it should be pointed out that these 13 patients with liver metastasis were all considered unresectable after MDT discussion, so they were enrolled in this clinical study.
Changes in the text: We have revised the sentence " the enrolled patients were not thoroughly screened" (lines in 458-459) and we deleted the sentence " patients enrolled were usually very complex.." because it was misexpressed (lines in 415-416). We added the fifth point to the inclusion criteria reinterpreting the inclusion criteria for patients with liver metastases (marked with red color).

Minor comments:
1. Key findings: Fruqintinib, exerted a certain degree of antitumor activity.
Reply : For this key finding, we concluded from our study that fuquinitinib combined with toripalimab produced better efficacy in patients with refractory recurrent metastatic colorectal cancer.
Changes in the text: We revised this sentence in the revised manuscript.
2. last sentence in this section: " ... pinpoint independent the prognostic variables..."
Reply : We would like to indicate that future studies need to identify prognostic variables in patients with advanced colorectal cancer with MSS receiving posterior line therapy.
Changes in the text: No change in the revised manuscript.
3. Line 92, is toripalimab really approved as a curative drug for resectable or metastatic melanoma?
Reply : We have to admit that immunotherapy has a good effect on melanoma. Numerous studies have demonstrated that PD-1 mab can even cure this malignant tumor for resectable or metastatic melanoma. Therefore, we are interested in

exploring the effect of the combination therapy on MSS patients with advanced colorectal cancer.
Changes in the text: No change in the revised manuscript.
4. line 107, fruqutininb is a part of brand-new generation...
Reply : We have checked this sentence and revised it in the manuscript (marked with red color).
Changes in the text: We have revised it in the revised manuscript.
5. lines 135-146, change to I, II, III etc to 1, 2, 3...
Reply : Considering the Reviewer's suggestion, We changed I, II, III etc to 1, 2, 3.
Changes in the text: The I, II, III etc were changed to 1, 2, 3 in the revised manuscript （marked with red color）.

Reviewer B

The authors draw conclusions which support advantage and safety of combination therapy of fruquintinib in combination with toripalimab. As mentioned in line 356, sample size is small, but indicated data are clear with tolerable toxicity. There are several manuscripts which indicate advantage and safety of combination therapy of fruquintinib in combination with programmed death-1 (PD-1) inhibitors. Therefore, description of the merit of toripalimab in compared with fruquintinib compared to other PD-1 inhibitors is essential. However this point is not clear. There are several anti-VEGFR agents, but all agents required hospitalization, except for only fruquintinib. The combination therapy expressed in this study is promising one. Please explain the merit why to select toripalimab.
Reply : We chose toripalimab as one of the combination agents for the following reasons: first, as discussed in the paper, Toripalimab has exhibited remarkable antitumoral activity in multiple solid tumors and was approved by the National Medical Product Administration (NMPA) in 2018 as a curative drug for unresectable or metastatic melanoma (lines 163-170); second, the US Food and Drug Administration (FDA) designated toripalimab as an orphan drug for the treatment of refractory advanced solitary malignant tumors (lines 171-173); third, Toripalimab has also demonstrated a similar response rate to pembrolizumab or nivolumab as a monotherapy in many preclinical studies and phase Ib/II clinical trials for several cancer types (lines 174-176); forth, there were no studies on the combination of toripalimab and fuquinitinib in the treatment of relapsed refractory metastatic colorectal cancer prior to our study.
Changes in the text:no change in the revised manuscript.

Reviewer C

Line 29: Remove the "t"

Reply : In the revised manuscript (version of “JGO-23-108-R1-4.6.docx”),we have already deleted the sentence, so we didn’t make any change.

Line 52: Can comparison to fruquintinib montherapy be provided in the abstract conclusions?

Reply: The conclusion of our study support the combination therapy of fruquintinib with toripalimab have the better effect than the fuquinitinib alone, so we changed our conclusion.

Change: In revised manuscript, we revised this sentence with marked red color

Line 61 (Highlight Box): How are authors concluding that "toripalimab confers benefit in prolonging the PFS and OS..." Presumably they mean over fruquintinib monotherapy, however, comparison was not made in this study, and this cannot be concluded by cross-trial comparison to the monotherapy trial. Also in the bullet point about "independent prognostic factors", were the authors intending to say that primary lesion excision and peritoneal metastasis were "predictive of benefit to the combination" not necessarily prognostic on their own?

Reply: We revised the sentence in line 61, the use of this combination therapy in patients with MSS mCRC is safe. Additionally, no significant toxicities of immunotherpy were observed. Responders to therapy demonstrated a survival benefit trend in this small and heterogeneous cohort. For the bullet point about "independent prognostic factors", we meant that primary lesion excision and peritoneal metastasis were predictive of benefit to the combination, we revised it in the revised manuscript (marked with red color).

Line 69: Surgery does not typically play a role unless aiming for curative surgical resection in metastatic disease.

Reply: We also acknowledge that surgery can make some sense for potentially curable metastatic disease, so surgery may be recommended for patients who achieve NED

Line 72: use of anti-EGFR therapy also depends on sidedness which should be mentioned

Reply: In revised manuscript, we revised this sentence with adding the description of tumor site (marked with red color).

Line 139: Was anti-EGFR mandatory if RAS wt or did it depend on RAS status and also sidedness?

Reply: The use of anti-EGFR therapy depends on RAS status and tumor site in the clinical practice. cetuximab is also an option for second - and third-line treatment in RAS wild-type mCRC patients.

Line 194: Not all RAS wt patients were treated with cetuximab. Why was that? Sidedness?
Reply: Of the 19 patients included in our study, 16 patients underwent RAS gene testing, of which 8 were RAS wild-type and 2 patients did not receive cetuximab because the toxic side effects.
Line 218: Very few patients did not get excision of primary tumor, so it would be hard to draw any conclusion based upon small number.
Reply: Because of the small sample size included in the study, we have to admit that it would be hard to draw a conclusion that patients with excision of primary tumor had a benefit of free-progressive survival. Subsequent studies for the expanding sample size are ongoing.
Line 284: For the sentence "In contrast to the North American..." it is hard to understand what point the authors are trying to make here. What line was REGONIVO used in and was this study different in that regard?
Reply: For the sentence "In contrast to the North American...", it meant that compared to “REGONIVO” study in North America, our study concluded that combination therapy had a higher response rate for the third-line MSS patients with mCRC. The REGONIVO study is also a study on third-line treatment of MSS type colorectal cancer, which isn’t different from our study.
Line 287: For the sentence "The REGONIVO study was a phase Ib...", all studies have strict I/E criteria, so it is not clear what point the authors are trying to make here about participants being carefully chosen and how this is contrasted with the current study.
Reply: Because the REGONIVO study was a phase Ib dose-escalation and dose-expansion trial, patients enrolled in the REGONIVO study were preferred, the final assessment indicator of the study, such as ORR, was superior to that of our study.
Line 305: This sentence is weak in claiming that "no additional evidence to exclude...pseudoprogression". Could it just be that there was a bit of progression before there was stability?
Reply: The imaging findings of patient No. 6 after the initial treatment suggested possible progress. We did not consider the progress of the disease in combination with the patient's clinical findings, so we assessed the overall efficacy of the patient as stable.
Line 316: Do the authors mean "randomized"?
Reply: We checked the sentence, it meant “in the randomized trial”.
Line 343: Other studies with anti-VEGF and anti-PD-1/PD-L1 have shown more benefit without liver mets. Was this seen in this study? How many patients without liver mets were treated and what was the response rate in these?

Reply: In our study, there were 6 patients without liver metastasis, among which 3 patients had lung metastasis, 2 patients had pelvic metastasis, and 1 patient had retroperitoneal metastasis. After receiving combination therapy, 2 patients had PR, 1 patient had SD, and 3 patients had PD, with ORR of 33% and DCR of 50%
Chang in the paper: we have descriped the therapy effect for liver metastasis patients in the revised manuscript (lines 313-316, marked with red color).
Line 358: What does it mean "not thoroughly screened"?
Reply: We checked this sentence and revised it (marked with red color).
Table 1: Is it possible to delineate "liver only" or "peritoneal only" as sites of disease?
Reply: Due to the small sample size, only patients with liver or lung metastases could not be shown in Table 1.

Reviewer D

1. First, the title needs to indicate efficacy and safety. .

Answer: According to the first suggestion, we have indicated efficacy and safety in the title.

2. Second, the abstract needs some revisions. The background did not indicate the clinical needs for this research focus and what the knowledge gap is on the efficacy and safety of Toripalimab + fruquintinib. The methods did not describe the inclusion of subjects, the assessment of baseline clinical factors, follow up procedures, and measurements of efficacy and safety outcomes. The results need to provide more detailed data on the safety outcomes.

Answer: Following the second comment, we have corrected the abstract and marked with the red color. First, the revised draft increases the clinical need for this study in the treatment of recurrent refractory MSS colorectal cancer. Second, in the method section, the revised draft added the assessment of baseline clinical factors, follow up procedures, and measurements of efficacy and safety outcomes, however, the inclusion of subjects had been described previous and marked with green color in the revised paper.

3. Third, the introduction of the main text, the authors need to have a detailed review on available third-line treatments for mCRC, analyze their limitations in efficacy and safety, analyze the potential reasons for the limited efficacy, describe the mechanisms of Toripalimab + fruquintinib, explain why the two treatments together is effective and safe, and clearly indicate the knowledge gaps.

Answer: Considering the Reviewer's suggestion, we modified the background part of the article. The modified content includes a detailed review on available third-line treatments for mCRC, analysis their limitations in efficacy and safety, analysis the potential reasons for the limited efficacy, description the mechanisms of Toripalimab

+ fruquintinib, explaination why the two treatments together is effective and safe, and clearly indication the knowledge gaps. These modification marked with red color in the revised manuscript.

4. Fourth, in the methodology of the main text, please describe the clinical research design, sample size estimation, and follow up details. In statistics, please describe the details of multiple Cox regression analysis.

Answer: As for the methodology of the main text, we have added descriptions of study design, sample size estimation, and follow-up details in our revised manuscript. For statistics, We reintroduced the specific analysis process and application of multi-factors COX regression analysis in detail in the revised manuscript.

5. Finally, please consider to cite the below papers to enrich the introduction and discussion of this paper

Answer: After careful reading of the article recommended by your journal, I think the study peformed by Li RR is suitable for reference, and I have quoted this article in the background of our manuscript.

Reviewer E

1. Please check the full name of "MSI-H" in the abstract and your main text. Which one is correct? Please unify.

26 **Background:** The most effective treatment with immune checkpoint inhibitors (ICIs)
27 is limited to the microsatellite instability (MSI-H) subgroup of advanced colorectal

41 with anti–programmed death-1 (PD-1) antibody toripalimab after standard treatment in
42 Chinese patients with non-microsatellite instability high (MSI-H)/mismatch repair
43 proficient (pMMR) mCRC.

120 for colorectal cancer and new treatment strategies are needed. Indeed, the immune
121 checkpoint inhibitors have shown efficacy only in a subset of patients with mCRC who
122 are mismatch repair–deficient or have a high level of microsatellite instability (MSI-H),
123 whose objective response rate (ORR) may reach 65% (7). Importantly, patients with
124 CRC and MSI-high (MSI-H) status generally show good response to immunotherapy

2. Please check all abbreviations in the main text, such as "DC", "DCR" below. All abbreviated terms should be full when they first appear.

184 (10,15). Targeting VEGF agents block the inhibitory signal transduction during DC
185 differentiation and reduce overall pool of MDSC (16).

(11.1% vs. 4.9%), DCR was 62.2% (28/45), median PFS equal 3.8 months, and median OS was 14.9 months.

3. Please indicate the specific institution name of "our hospital".

##Study design and participants

This study was a single-arm, single-center, prospective, phase II trial or which patients were recruited from our hospital. From December 2019 to August 2022, we

4. "Galle" is not the author of reference 23 and "Dai" is not the author of reference 24. Please check and revise.

with avelumab plus axitinib and 25.5% with sunitinib. Galle et al. has announced that the combination of atezolizumab and bevacizumab demonstrated superior OS (19.2 months vs. 13.4 months, P=0.0009) and PFS (6.9 months vs. 4.3 months, P<0.001) compared to sorafenib in the first-line treatment of advanced HCC (23). Data from

angiogenesis. In 2022 Dai et al (24) reported the efficacy of fruquintinib in combination with PD-1 inhibitors in patients with refractory non-MSI-H/pMMR metastatic

23. Finn R, Qin S, Ikeda M, et al. IMbrave150: Updated overall survival (OS) data from a global, randomized, open-label phase III study of atezolizumab (atezo) plus bevacizumab (bev) versus sorafenib (sor) in patients (pts) with unresectable hepatocellular carcinoma (HCC). JOURNAL OF CLINICAL ONCOLOGY 2021;39.

24. Gou M, Qian N, Zhang Y, et al. Fruquintinib in Combination With PD-1 Inhibitors in Patients With Refractory Non-MSI-H/pMMR Metastatic Colorectal Cancer: A Real-World Study in China. Front Oncol 2022;12:851756.

5. Please check if any more references need to be added in the below 2 sentences since you mentioned "Studies", but only one reference was cited. If not, "studies" should be changed to "a study/a previous study".

Toripalimab has also demonstrated a similar response rate to pembrolizumab or nivolumab as a monotherapy in many preclinical studies and phase Ib/II clinical trials for several cancer types (36). Recent studies have identified that simultaneous

metastases was relatively small. Third, several studies suggested that tumor mutation burden (TMB), PD-L1 expression, circulating tumor DNA (ctDNA) levels, tumor-infiltrating lymphocytes (TILs), gene expression profiling (GEP) of an inflammatory microenvironment, and neoantigen prediction have become independent predictors of immunotherapy in multiple solid tumors (57); however, these were not evaluated in our

6. Table 1:

The data below in your main text is inconsistent with Table 1. Please check.

(15.7%) with raltitrexed.13 patients had gene mutation testing and 8 patients have mutations in RAS oncogene. The patient characteristics are detailed in Table 1.

Primary lesion resected	15 (78.95)
***KRAS* mutant (n=16)**	8 (50.00)

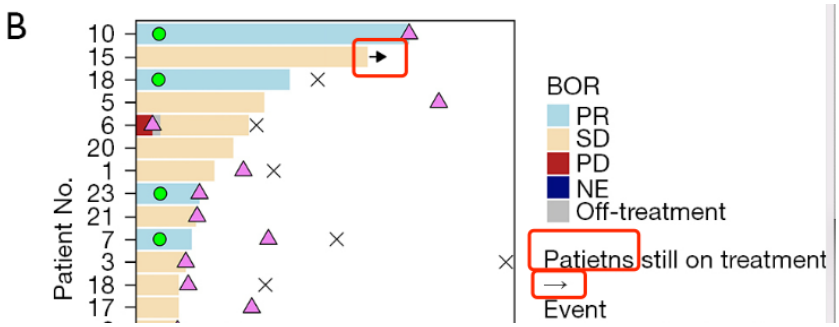
7. Please unify "KRAS" and "RAS" in your whole text and your Tables.

***KRAS* mutant (n=16)**	8 (50.00)	
***RAS* mutant**	1.36 (0.38–4.88)	0.635

8. Figure 1:

1) Please revise "BoR" to "BOR" and indicate the full name of "BOR" in the legend.

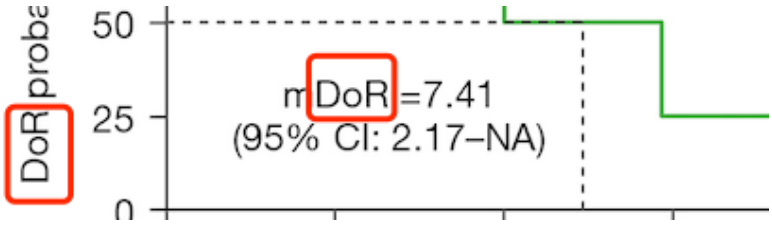
2) Please unify the arrow in Figure 1B and there is a spelling mistake "patietns". Please revise.



3) Please indicate the full name in legend for all the abbreviated terms appearing in Figure 1 such as "PR", "SD", “PD", etc.

9. Figure 2:

1) Please revise “DoR” to “DOR”.



2) Please indicate the full name in legend for all the abbreviated terms appearing in Figure 2 such as "mPFS", "mOS", “mDOR", “NA”, etc.

3) Your Figure 2 legend don’t match with your Figure 2. Figure 2A is OS, not PFS.

958 Figure 2 PFS, OS and DOR curves of patients with advanced CRC treated with
959 toripalimab and fruquintinib (A) The median progression-free survival of the
960 combined treatment, (B) the median overall survival of the combined treatment, and