Peer Review File

Article information: https://dx.doi.org/10.21037/jgo-23-590

Reviewer A

1) First, the title needs to be specific to a retrospective cohort study

Reply 1: Thanks for very much your comment. We changed the title to Efficacy and Safety of Regorafenib in Combination with Immune Checkpoint Inhibitor Therapy as Second-Line and Third-Line Regimen for Patients with Advanced Hepatocellular Carcinoma: a retrospective study

Changes in the text: see Page 1, line 2

2) Second, the abstract needs further revisions. The background did not explain why regorafenib plus ICIs is potentially effective and safe. The methods did not describe the inclusion of subjects, the assessment of baseline clinical characteristics, follow up procedures, and measures of efficacy and safety. The results need to briefly summarize the baseline characteristics of the patient cohort. The current conclusion needs to be tone down since this is not a RCT.

Reply 2: Thanks for very much your comment. We add the feasibility analysis of regorafenib combined with ICI in the background section of the abstract. The relevant information in the methods and results section has been supplemented. We have revised in the conclusion section of the abstract as you suggested, as following:

Regorafenib combined with PD-1 inhibitor is a promising regimen in treating patients with advanced HCC owing to its safety and effectiveness as well as low incidence of serious adverse events with its use.

Changes in the text: see Page 2, line 45, 55, 64, 74

3) Third, in the introduction of the main text, the authors need to review what has been known on the treatment of patients with advanced hepatocellular carcinoma following the failure of combined tyrosine kinase inhibitor plus immune checkpoint inhibitor therapy, and analyze why regorafenib plus ICIs is potentially effective and safe.

Reply 3: Thanks for very much your comment. We add a prognostic presentation for patients with advanced hepatocellular carcinoma after the failure of tyrosine kinase inhibitors combined with immune checkpoint inhibitors, and we add a feasibility analysis for regorafenib combined with ICIs as follows:

However, the efficacy of the combination remains suboptimal, with an ORR of lower than 40% and a median progression-free survival (PFS) of almost 6 months (12-14). Regorafenib is a VEGFR inhibitor, which could increase PD-L1 expression in tumors and increase intratumoral CD8+ T-cell infiltration by normalizing the cancer vasculature and improving the efficacy of the PD-1 antibody (15,16). Regorafenib is the only systemic treatment shown to

provide survival benefit in HCC patients progressing on sorafenib treatment (17). All these provide the theoretical basis for the combined strategy of regorafenib and PD-1 inhibitors.

Changes in the text: see Page 4, line 122 and Page 5, line 128-134,

4) Fourth, in the methodology of the main text, please accurately describe the clinical research design, sample size estimation procedures, data collection of baseline clinical characteristics, and follow up procedures. In statistics, please describe the groups to be compared by using log-rank test and ensure $P<0.05$ is two-sided.

Reply 4: Thanks for very much your comment. We have supplemented it in the methods section of the article according to your suggestion, as following:

We reviewed the electronic medical records of consecutive patients with advanced HCC who had failed previous treatment with TKIs (at least 1 TKI including apatinib, sorafenib, or lenvatinib) combined with PD-1 inhibitors and were treated with regorafenib and a PD-1 inhibitor (camrelizumab or sintilimab) from November 15, 2020, to January 31, 2022 in the First Affiliated Hospital of Nanjing Medical University.

HCC was diagnosed according to the European Association for the Study of Liver and American Association for the study of Liver Disease guidelines. The inclusion criteria for the study population were as follows: 1) age ≥ 18; 2) Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; 3) radiographic disease progression on first-line treatment with apatinib, sorafenib or lenvatinib. Patients were excluded from this study if they: 1) participation in other interventional clinical studies during regorafenib administration; 2) unavailable data for study analysis; 3) presence of other primary solid tumors or hematologic malignancies.

As is standard practice at our institution, regorafenib was given orally at a dose of 40, 80, or 120 mg/m2/day, days 1–21) every 4 weeks. Standard doses of anti-PD-1 antibodies (camrelizumab 200 mg or sintilimab 200 mg) were administered intravenously every 3 weeks. Clinical and laboratory data were retrospectively collected from the eligible patients. The patients' demographics was also collected.

According to standard practice, patients were followed-up every 6–8 weeks using computed tomography (CT) or magnetic resonance imaging (MRI) of the chest, abdomen, pelvis, and other known sites of disease.

Changes in the text: see Page 5, line 143 -148, line 151 -169

5) Finally, please consider to review and cite several related papers: 1. Uson Junior PLS, Liu AJ, Sonbol MB, Borad MJ, Bekaii-Saab TS. Immunotherapy and chimeric antigen receptor T-cell therapy in hepatocellular carcinoma. Chin Clin Oncol 2021;10(1):11. doi: 10.21037/cco-20-231. 2. Sahin IH, Khalil L, Millett R, Kaseb A. Neoadjuvant and adjuvant treatment approaches for hepatocellular carcinoma: future outlook. Chin Clin Oncol 2021;10(1):7. doi: 10.21037/cco-20-248. 3. Zou J, Huang P, Ge N, Xu X, Wang Y, Zhang L, Chen Y. Anti-PD-1 antibodies plus lenvatinib in patients with unresectable hepatocellular carcinoma who progressed on lenvatinib: a retrospective cohort study of real-world patients. J Gastrointest Oncol 2022;13(4):1898-1906. doi: 10.21037/jgo-22-643.

Reply 5: Thanks for very much your comment. We have consulted and cited the literature you mentioned.

Changes in the text: see Page 4, line117, and Page 9, line 258

Reviewer B

The paper titled “Efficacy and safety of regorafenib plus immune checkpoint inhibitor in patients with advanced hepatocellular carcinoma following the failure of combined tyrosine kinase inhibitor plus immune checkpoint inhibitor therapy: a retrospective study” is interesting. Regorafenib combined with PD-1 inhibitor was well-tolerated with manageable safety profile and exhibited positive antitumor activity. This regimen might be a safe treatment option for advanced HCC patients that are progressing on TKI plus ICI regimens. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) In the introduction of the manuscript, it is necessary to clearly indicate the characteristics and evaluation criteria of immunotherapy and the impact of immunotherapy on tumor micrometastasis.

Reply 1: Thanks for very much your comment. We have made a supplement in the introduction section of the article as you suggested, as following:

Tumor cells may evade detection by the immune system by upregulation of cell-surface immune checkpoints, while checkpoint inhibitors function by unmasking these cells and potentiating immune responses against them and have been utilized in a growing number of HCC and other solid tumors (5). In addition,using adjuvant/neoadjuvant ICIs or other ICIs combination therapies can reduce HCC recurrence, eliminate micrometastatic occurrance and improve patients' prognosis (6,7).

Changes in the text: see Page 4, line 114-120

2) What are the predictors of efficacy of immunotherapy? What is the application value of PD-1 inhibitors in neoadjuvant treatment of hepatocellular carcinoma? It is recommended that relevant information be added to the discussion.

Reply 2: Thanks for very much your comment. We have made a supplement in the discussion section of the article as you suggested, as following:

Cancer immunotherapies, such as ICIs, which is capable of inducing the immune system to effectively recognize and attack tumors, can be affected by several parameters/predictors, including tumour mutational burden (TMB), epigenetic modifications, the degree of mismatch repair deficiency (MMR-D) and microsatellite instability (MSI) (18,19). Besides, ICIs can be used as neoadjuvant or adjuvant treatment approaches for HCC and other cancers (5).

Changes in the text: see Page 9, line 250-255

3) With the discovery of new drug targets and the continuous emergence of new combination treatment options, what breakthroughs will there be in the treatment of hepatocellular carcinoma in the future? What inspiration can this study provide? It is recommended to add relevant content to the discussion.

Reply 3: Thanks for very much your comment. We believe that with the discovery of the continuous emergence of new drug targets and new combined treatment regimens, the choice of TKI drugs is very critical for patients with advanced HCC, especially for those who need to receive second-line or third-line immunotherapy. Our study found that PD-1 inhibitor combined with regorafenib is a promising regimen in treating patients with advanced HCC owing to its safety and effectiveness as well as low incidence of serious adverse events with its use. Our study provides a therapeutic reference for more patients with advanced HCC. At the same time, our next research plan involves finding relevant indicators that affect the prognosis and efficacy of patients with advanced HCC and screening biomarkers for patients who can benefit from PD-1 inhibitor combined with regorafenib in order to achieve personalized precision treatment for patients with advanced HCC.

Changes in the text: see Page 11, line 330-338

4) The fonts need to be enlarged as shown in Figures 1 and 2.

Reply 4: Thanks for very much your comment. We have finished the modification according to your suggestion.

5) What are the advantages of combination therapy? It is recommended to add relevant comparative analysis.

Reply 5: Thanks for very much your comment. We have added the advantages of combination therapy in the discussion section and cited a study comparing ICIs therapy with combination therapy, as following:

Combination therapy with TKI and ICIs can normalize tumor blood vessels and improve the efficacy of ICIs, increase the expression of PD-L1 in tumors, and increase the infiltration of CD8+ t cells in tumors, thus increasing the benefits of patients (16). In the ORIENT-32 trial, the combination of the PDL1 inhibitor sintilimab with a bevacizumab biosimilar proved superior to sorafenib among 571 HCC patients. After a median follow-up of 10 months, median overall survival was 10.4 months in the sorafenib arm and was not reached in the combination arm (HR 0.57, 95% CI 0.43-0.75; $P < 0.0001$) (23).

Changes in the text: see Page 9, line 258-265

6) What are the highlights and significance of this study? What is the author's next research plan? It is recommended to add relevant content to the discussion.

Reply 6: Thanks for very much your comment.

In China, 39.0%-53.6% of patients with HCC have been diagnosed at an advanced stage and have lost the opportunity of radical treatment. At this time, immunotherapy combination therapy becomes extremely important. However, the objective response rate (ORR) remains

very limited for advanced HCC regardless of the modality of immunotherapy combination. Hence, the choice of TKI drugs is very critical for patients with advanced HCC, especially for those who need to receive second-line or third-line immunotherapy. In our study, we found that, the confirmed ORR and DCR were 46.2% and 69.2% for patients receiving second-line treatment, respectively. At the same time, the results show that there are 3 cases (17.64%) in patients with grade 3 AE, but no cases of grade 4 AE. PD-1 inhibitor combined with regorafenib is a promising regimen in treating patients with advanced HCC owing to its safety and effectiveness as well as low incidence of serious adverse events with its use. In the future study, we plan to conduct prospective, interventional studies with larger sample size to further confirm the efficacy and safety of PD-1 inhibitor combined with regorafenib. At the same time, we will involve finding relevant indicators that affect the prognosis and efficacy of patients with advanced HCC and screening biomarkers for patients who can benefit from PD-1 inhibitor combined with regorafenib in order to achieve personalized precision treatment for patients with advanced HCC.

Changes in the text: see Page 11, line 329

7) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Hepatic arterial infusion chemotherapy and immune checkpoint inhibitors, alone or in combination, in advanced hepatocellular carcinoma with macrovascular invasion: a single-centre experience in Taiwan, J Gastrointest Oncol, PMID:37201085”. It is recommended to quote this article.

Reply 7: Thank you for your valuable suggestion. We have cited related literature in the proper place of the revised manuscript.

Changes in the text: see Page 4, line 118-120

8) What is the tumour- and class-specific patterns of immune-related adverse events of immune checkpoint inhibitors? It is recommended to add relevant content.

Reply 8: Thank you very much for your advice. We have supplemented this in the discussion section of the article, as following:

The increasing use of combination strategies (combining immunotherapy with traditional therapies such as chemotherapy or TKI) may improve the efficacy of cancer immunotherapy, but may also amplify immune related adverse events (irAEs) (28). Different immune microenvironments may drive tissue-specific irAE patterns. There are various irAEs in trials using ICI drugs. The endocrine, skin, and gastrointestinal systems are most commonly affected by irAEs (29,30). Other tumor dependent irAE spectra can be determined from ICI experimental data (31).

Changes in the text: see Page 10, line 299-305

Reviewer C

1. Please check all abbreviations in the abstract and the main text, such as ORR in Introduction. Abbreviated terms should be full when they first appear.

Reply: Thank you very much for your comment. We have checked and revised.

2. Ref. 15 and Ref. 40 are the same. Please check and revise.

Reply: Thank you very much for your comment. We have checked and revised.

3. Ref. 17 and Ref. 32 are the same. Please check and revise.

Reply: Thank you very much for your comment. We have checked and revised.

4. Table 2

The data (red box) look confusing. Please check and confirm whether % should be unified for all data in these four boxes.

Deaths	1 (5.88%)
Survivors	16 (94.12%)
95% CI	10.02, –
OS rate (%)	
6 months (95% CI)	100.00% (100.00–100.00%)
9 months (95% CI)	100.00% (100.00–100.00%)
12 months (95% CI)	83.33% (27.31–97.47%)
15 months (95% CI)	83.33% (27.31–97.47%)
Median PFS (months)	5.09 (2.92–12.42)
ORR (%)	41.2
DCR (%)	64.7
Median follow-up time (months) (95% CI)	7.62 (4.76–10.28)

Reply: Thank you very much for your comment. We have revised, as following:

Outcome	N=17
Deaths	1 (5.88%)
Survivors	16 (94.12%)
95% CI	10.02, –
OS rate (%)	
6 months (95% CI)	100.00% (100.00–100.00)
9 months (95% CI)	100.00% (100.00–100.00)
12 months (95% CI)	83.33% (27.31–97.47)
15 months (95% CI)	83.33% (27.31–97.47)
Median PFS (months)	5.09 (2.92–12.42)
ORR (%)	41.2
DCR (%)	64.7
Median follow-up time (months) (95% CI)	7.62 (4.76–10.28)

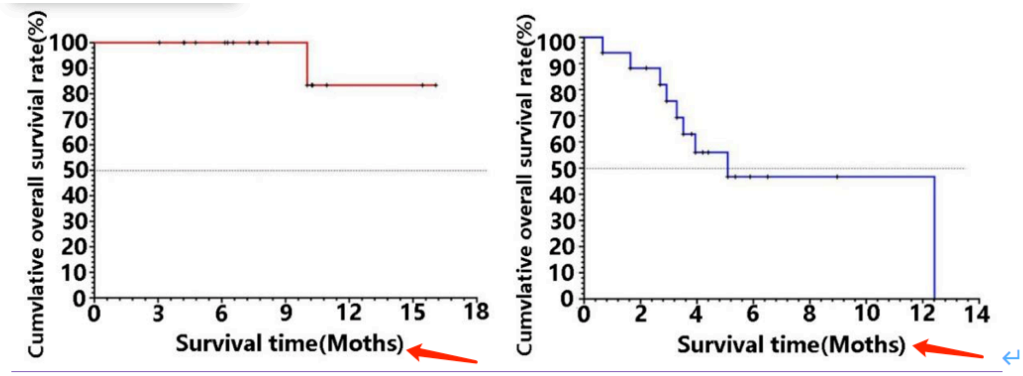
5. Figure 1

There seems to be no “PFS, OS, PD-1” in Figure 1, while it was explained in the legend. Please check and revise.

Reply: Thank you very much for your comment. We have checked and revised.

6. Figure 2

Please revise this typo.



Reply: Thank you very much for your comment. We have checked Figure 2 and revised.