Peer Review File

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Reviewer #A

This is a single-center, retrospective, real-world study assessing the effectiveness of combination therapy with regorafenib plus PD-1 inhibitor in the second-line setting for patients with advanced HCC who were treated with second-line regorafenib combined with a PD-1 inhibitor or regorafenib monotherapy were evaluated. The progression-free survival (PFS), objective response rate (ORR), and disease control rate (DCR) were observed.

The study included forty-six patients with HCC, most of whom had received previous systemic treatment including targeted therapy and immunotherapy. Tumor response was evaluated in 25 patients in the regorafenib plus PD-1 inhibitor group and 21 patients in the regorafenib monotherapy group: the ORR was 21.7% and 8.7%, and the DCR was 47.8% and 32.6%, respectively. The median PFS of the regorafenib plus PD-1 inhibitor group was 11.5 months, which was significantly longer than that of the regorafenib monotherapy group (5.1 months) (P=0.049).

The authors concluded that regorafenib combined with a PD-1 inhibitor provided significant clinical benefit for HCC patients with progression after first-line treatment supporting the need for further analysis in a real-world study with a large cohort to substantiate their findings.

The study is of clinical interest since the choice of second-line treatments after first-line failure is currently a very important topic in the HCC setting.

I have only a few comments.

-the combination treatment strategy is now a very promising approach in HCC systemic treatment. The authors should further discuss why this combined treatment approach is demonstrating increasing anti-tumor efficacy. In particular, it has been recently reported that some TKIs have synergistic effects with immune checkpoint inhibitors due to their effect on immune cells, as recently described in a comprehensive review (TKIs in combination with immunotherapy for hepatocellular carcinoma. Expert Rev Anticancer Ther. 2023 Mar;23(3):279-291. doi: 10.1080/14737140.2023.2181162.). In particular, among TKIs, it has been reported that Regorafenib demonstrates anti-immunosuppressive properties, as well as promoting anti-tumor immunity by modulating macrophages and increasing the proliferation and activation of CD8+ T cells, as recently well-described (Experience with regorafenib in the treatment of hepatocellular carcinoma. Therap Adv Gastroenterol. 2021 May 28;14:17562848211016959. doi: 10.1177/17562848211016959.).

Reply: We sincerely appreciate your detailed review and suggestions, which have been instrumental in enhancing our manuscript. Regarding the combination treatment strategy for HCC, we concur with your observation about the rising promise of this approach. We've incorporated the insights you highlighted into our manuscript to provide a more in-depth discussion.

Changes in the text: The emerging strategy of combining treatments is gaining prominence in HCC systemic therapy. This approach's growing anti-tumor efficacy is rooted in the synergistic effects of some tyrosine kinase inhibitors (TKIs) with immune checkpoint inhibitors (1). It is crucial to delve deeper into the mechanistic underpinnings that drive the

impressive anti-tumor efficacy of the regorafenib plus PD-1 inhibitor combination therapy. Recent studies have shed light on the intricate crosstalk between the immune system and regorafenib's unique properties. Specifically, regorafenib showcases anti-immunosuppressive properties, promoting anti-tumor immunity. It achieves this by modulating macrophages and augmenting the proliferation and activation of CD8+ T cells (2). As we continue to explore therapeutic frontiers for HCC, integrating such potent combinations stands as a beacon of promise in optimizing patient outcomes (Page 8, Line 215).

Reference:

1. Stefanini B, Ielasi L, Chen R, Abbati C, Tonnini M, Tovoli F, et al. TKIs in combination with immunotherapy for hepatocellular carcinoma. Expert Rev Anticancer Ther. 2023;23(3):279-91.
2. Granito A, Forgione A, Marinelli S, Renzulli M, Ielasi L, Sansone V, et al. Experience with regorafenib in the treatment of hepatocellular carcinoma. Therap Adv Gastroenterol. 2021;14:17562848211016959.

Reviewer #B

In a retrospective study, the authors reported that second-line regorafenib + PD-1 antibody combination therapy for advanced hepatocellular carcinoma was superior to regorafenib alone in both PFS and ORR. This is a very interesting and worthwhile study, but it contains several points that need to be resolved.

1) The evaluation of hepatic reserve capacity in both groups is not described at all. Liver reserve capacity has a significant impact on liver cancer treatment. This point needs to be clarified.

Reply: We appreciate the reviewer's keen observation on the absence of data regarding the hepatic reserve capacity of the patients in both groups. Indeed, the hepatic reserve capacity is a critical factor influencing liver cancer treatment outcomes. Based on our clinical experience, patients chosen for both treatments investigated in this study are usually not in an end-stage liver disease condition. Most have a considerably preserved liver function reserve. To supplement corresponding data of hepatic reserve capacity, we retrospectively reviewed the pre-treatment laboratory examination reports and observed that all the patients had Child-Pugh grade A. Given this context, it is supposed that patients in the two groups exhibited comparable results and shared common feature of hepatic reserve capacity. The potential confounding impact of the hepatic reserve capacity on the comparison analysis of the two groups should be considered minor or even nonexistent. We thank the reviewer for pointing this out, and we have added the additional data of Child-Pugh grade in the revised manuscript.

Changes in the text: In the Results and Table 1 of the revised manuscript (Page 6, Line 157).

2) Regorafenib is a drug that has shown efficacy after sorafenib, but is sorafenib the pre-treatment TKI in this study? It should be clarified whether there is any bias toward pre-treatment agents, as some reports indicate poor efficacy of second-line therapy after lenvatinib.

Reply: Thank you for pointing out the need for clarity regarding the pre-treatment with

TKIs in our study. In our cohort, the majority of patients had been treated with sorafenib as their primary TKI prior to receiving regorafenib (61.9% in the regorafenib group and 72% in the combination group, respectively). We recognize the difference for patients in the two groups pre-treated with sorafenib and other target therapies was very small ($P > 0.05$). Therefore, the potential bias toward pre-treatment agents, while worth acknowledging, should be considered of a minor nature and may not demand a substantial adjustment. We have provided this information and corresponding description in our revised manuscript.

Changes in the text: In the Results and Table 1 of the revised manuscript (Page 6, Line 163).

3) There is no mention of VP. This point should also be evaluated to see if there is any bias between the two groups.

Reply: We appreciate the reviewer's attention to the potential influence of vascular invasion and VP on our findings. We have carefully considered your concerns and would like to address them as follows. In our study, we utilized the Cheng's classification for assessing vascular tumor thrombosis due to its prominence in Chinese literature and clinical practice. This classification has been shown to be suitable for assessing the condition, treatment selection, and prognosis of patients in China. As per the consensus recommendations in our region, we employed Cheng's classification to categorize vascular tumor thrombosis. We found that there were no significant differences in the distribution of PVTT classifications between the monotherapy and combination therapy groups. In the monotherapy group, the distribution of patients according to the Cheng's classification was as follows: I0 (47.6%), I (28.6%), II (5%), III (14.3%), and IV (5%). In the combination therapy group, the distribution was as follows: I0 (80%), I (12%), II (8%), III (0%), and IV (0%). Chi-square tests were conducted to analyze the data, and the results indicated that there were no significant differences in PVTT classification between the two groups ($P>0.05$). Based on these findings, we believe that the potential for bias is minimal in this aspect of our study. Once again, we appreciate your thoughtful review and constructive comments, which have been instrumental in improving the overall quality and rigor of our research. We have incorporated the added data in both the Results section and Table 1 of our manuscript, providing readers with a comprehensive understanding of our research.

Changes in the text: In the Results and Table 1 of the revised manuscript (Page 6, Line 166).