

Response to Reviewer Comments

Above all, thank you very much for your helpful comments on improving the quality of this article. We are grateful for your efforts in reviewing our paper and providing positive feedback. We have revised the manuscript and focused on addressing your comments and suggestions. Below, we will address your concerns.

Reviewer #1:

Point 1: Abstract: The results should include specific statistical values (e.g., "HR=0.7076") and clearly highlight the key findings from the subgroup analysis (e.g., benefits for male and right-sided colon cancer patients).

Response 1:

Thank you for your valuable suggestion. We have supplemented the Results section of the abstract with the relevant specific statistical values. Additionally, in the abstract's Conclusions section, we have highlighted the findings from the subgroup analysis, which provide robust real-world evidence to support the identification of optimal candidates for combination immunotherapy in future studies.

Results

No significant differences were observed in PFS (HR=0.7076, 95% CI: 0.4069-1.23, p=0.22) or OS (HR=1.154, 95% CI: 0.4712-2.827, p=0.75) between the two groups.

Subgroup analysis suggested that chemotherapy combined with bevacizumab and anti-PD-1 immunotherapy has potential benefits for male patients (HR=0.33, 95% CI: 0.14-0.81, p=0.025) and right-sided MSS mCRC patients (HR=0.40, 95% CI: 0.17-0.95, p=0.022).

Conclusions

Male patients or those with right-sided mCRC may derive benefit from the immune-based combination therapy.

Point 2: Introduction: Recent key clinical trials should be added to compare the treatment challenges faced by MSS patients.

Response 2:

Thank you for raising this important concern. We have incorporated the relevant references in the Introduction section, including the recent METIMMOX study on the efficacy of combination immunotherapy in MSS mCRC, with proper citations and corresponding discussions to contextualize their significance.

The METIMMOX study was a Phase II clinical trial comparing the efficacy of

chemotherapy combined with nivolumab versus chemotherapy alone as first-line treatment for MSS mCRC^[15]. Results showed a median PFS of 6.6 months in the combination immunotherapy group versus 5.6 months in the chemotherapy-alone group. This study suggested that short-course oxaliplatin-based chemotherapy in MSS mCRC patients may alter tumor immunogenicity, potentially inducing responsiveness to immune checkpoint inhibitors.

[15] REE A H, ŠALTYTĖ BENTH J, HAMRE H M, et al. First-line oxaliplatin-based chemotherapy and nivolumab for metastatic microsatellite-stable colorectal cancer-the randomised METIMMOX trial [J]. *Br J Cancer*, 2024, 130(12): 1921-8.

Point 3: The first paragraph of the discussion mentions methods for overcoming the generally poor efficacy of immunotherapy, but the explanation is insufficient. Numerous studies have shown that tertiary lymphoid structures (TLS) are important biomarkers in colorectal cancer (PMID: 39575712). One study indicated that mature TLS can predict the efficacy of immune checkpoint inhibitors in solid tumors, independent of PD-L1 expression (PMID: 35118423). Therefore, TLS has great potential in addressing this challenge.

Response 3:

We sincerely appreciate your valuable suggestion. We have added relevant content to the first paragraph of the Discussion section, including currently identified biomarkers that can predict the efficacy of immunotherapy. These biomarkers encompass tertiary lymphoid structures (TLS), Immunoscore-IC, CD8+ T cells, regulatory T cells, and M2 macrophages.

For instance, studies have demonstrated that Immunoscore-IC is currently the most promising biomarker for predicting therapeutic benefits from immune combination therapy^[21]. Notably, responders to immune combination therapy exhibited significantly higher densities of CD8+ T cells, regulatory T cells, and M2 macrophages compared to non-responders^[22]. Furthermore, extensive research has identified tertiary lymphoid structures (TLS) as key prognostic biomarkers in colorectal cancer^[23]. A notable study revealed that the presence of mature TLS can independently predict the efficacy of immune checkpoint inhibitors in solid tumors, irrespective of PD-L1 expression levels^[24]. Given these findings, TLS holds significant potential in overcoming current challenges in immunotherapy response prediction.

[21] MORETTO R, ROSSINI D, CATTEAU A, et al. Dissecting tumor lymphocyte infiltration to predict benefit from immune-checkpoint inhibitors in metastatic colorectal cancer: lessons from the AtezoT RIBE study [J]. *J Immunother Cancer*, 2023, 11(4).

[22] TAKEI S, TANAKA Y, LIN Y T, et al. Multiomic molecular characterization of the response to combination immunotherapy in MSS/pMMR metastatic colorectal cancer [J]. *J Immunother Cancer*, 2024, 12(2).

- [23] LV J, ZHANG X, ZHOU M, et al. Tertiary lymphoid structures in colorectal cancer [J]. Ann Med, 2024, 56(1): 2400314.
- [24] VANHERSECKE L, BRUNET M, GUÉGAN J P, et al. Mature tertiary lymphoid structures predict immune checkpoint inhibitor efficacy in solid tumors independently of PD-L1 expression [J]. Nat Cancer, 2021, 2(8): 794-802.

Point 4: There are numerous Chinese punctuation marks ("。") following citation numbers throughout the manuscript; please correct this. After addressing the above modifications, I believe this article is suitable for publication in the World Journal of Gastroenterology.

Response 4:

Thank you for your valuable suggestion. We have incorporated the recommended changes into the manuscript accordingly.

Reviewer #2:

Point 1: First, the sample size was relatively small, with only 25 patients remaining in the experimental group after matching. Such a small number reduces the statistical power of the study, making it harder to detect real differences between groups. In fact, when examining the Kaplan-Meier survival curves, there appeared to be a trend favoring improved PFS in the combination therapy group. Yet, this trend did not reach statistical significance, likely due to the limited sample size.

Response 1:

We acknowledge that our study included a small number of patients in the experimental group. Although we employed 1:4 propensity score matching to enhance statistical power and conducted multiple sensitivity analyses to verify the robustness of our findings, the limited sample size remains an inherent limitation of this study. We have expanded the Discussion section to explicitly address these limitations in interpreting the results. As you rightly pointed out, while there appeared to be a trend favoring improved PFS in the combination therapy group, this trend did not reach statistical significance—likely due to insufficient statistical power from the small sample size. Consequently, we cannot definitively determine whether the negative result reflects a true lack of efficacy or a false-negative outcome stemming from limited statistical power. Therefore, we emphasize the need for cautious interpretation of our conclusions and strongly advocate for further validation through large-scale prospective trials.

Additionally, this study was designed as a retrospective cohort analysis, which inherently carries risks of selection bias and unmeasured confounding factors that may influence the validity of the findings. While we adjusted for known confounders using statistical methods, we fully recognize that such adjustments cannot fully substitute for a RCT, particularly given the potential impact of unmeasured variables. Thus, the results should be interpreted with appropriate caution.

Our multicenter retrospective cohort study has several limitations that warrant consideration. First, the retrospective design with a small sample size inherently limits results robustness. Although the experimental group showed a trend toward improved progression-free survival (PFS) with immune combination therapy, it failed to demonstrate statistical significance, likely due to insufficient statistical power. These results should therefore be interpreted with caution and require validation in larger prospective studies.

Point 2: Another concern is the use of different anti-PD-1 agents—including penpulimab, pembrolizumab, and sintilimab—across patients, which could introduce variability in treatment effects and complicate interpretation of the results.

Response 2:

We sincerely appreciate your valuable feedback. As you rightly highlighted, the heterogeneity in chemotherapy regimens (FOLFOX, FOLFIRI, and CAPEOX) and the use of different PD-1 inhibitors in this study may lead to variations in treatment efficacy and complicate the interpretation of results. We considered addressing this issue through standardized treatment protocols, stratification based on chemotherapy regimens and specific immunotherapy agents, and subgroup analyses. However, the small sample size in the experimental group made these approaches unfeasible at this stage. We have explicitly acknowledged this limitation in our study's limitations section. In our future study design, we plan to use a uniform chemotherapy regimen and the same PD-1 inhibitor to compare the efficacy between the two groups, thereby minimizing the confounding effects of different drug types on the trial outcomes.

Third, therapeutic heterogeneity may have impacted our findings. The study incorporated multiple chemotherapy regimens (FOLFOX, FOLFIRI, and CAPEOX) and five different anti-PD-1 agents, creating substantial variability that could obscure true efficacy comparisons between treatment approaches.

Point 3: Overall, while the study offers encouraging early signals, larger, prospective, and more standardized studies are needed to validate these findings and better define the role of anti-PD-1 therapy in this patient population.

Response 3:

Thank you for your valuable suggestion. Our team is currently preparing to launch an investigator-initiated trial, conducting a multicenter randomized controlled study. Currently, we are in the process of ethical approval applications, securing research funding, and coordinating with participating centers. We aim to provide higher-level evidence to better define the role of combination immunotherapy in MSS mCRC.

We are very grateful for your and the reviewers' warm work earnestly. In all, we found the reviewers' comments are quite helpful. They point out the deficiencies in our manuscript and help us with further improvement.

We have tried our best to improve the manuscript and made extensive modifications to the original manuscript according to the comments. These changes will not influence the content and framework of the paper. Here did not list all the changes but marked them in yellow in the revised manuscript.

Revision reviewer 1

Comment: Accept

Reply: Thanks for your comments.