Manuscript ID: NO: 115009

Title: Molecular Profiling-Directed Individualized Adjuvant Therapy in Colorectal Cancer: Bridging Consensus Guidelines to Clinical Disparities

Dear Editor,

We thank you and the anonymous reviews very much for the constructive criticism. Please find below the amendments that we have made in this revision. I will be glad to receive further editorial correspondence through this email: xfcheng@zju.edu.cn

Best regards,

Dr. Xiaofei Cheng

Responds to the reviewer 1's comments:

1. Response to comment: "*First, the review would benefit from greater methodological transparency befitting a state-of-the-art narrative synthesis. Please add a brief Methods paragraph describing how the literature was identified and appraised: databases searched, date limits, language restrictions, core inclusion priorities (adjuvant setting, perioperative trials, real-world evidence), and how conflicting evidence was reconciled.*"

Response: We sincerely thank the reviewer for this critical suggestion to enhance the methodological rigor of our review article. As recommended, we have now added a dedicated paragraph in the Introduction section to explicitly detail the literature search strategy, including the databases searched, inclusion/exclusion criteria, and the time range covered. This addition ensures full transparency and reproducibility of our evidence synthesis process. The new text is highlighted in yellow and has been integrated into the revised manuscript.

2. Response to comment: "*Because several recommendations hinge on the distinct roles of prognostic versus predictive biomarkers, the paper should also standardize evidence labeling in text, tables, and figures. For example, ctDNA is*

positioned as a driver of adaptive therapy, yet current guidelines largely confine its use to trial contexts; a compact table listing assay types, timing windows, positivity thresholds, and performance metrics would sharpen claims and help clinicians interpret platform variability."

Response: We sincerely thank the reviewer for this critical and constructive observation. We fully agree that clearly distinguishing between prognostic and predictive biomarkers is essential for accurate interpretation of our recommendations, and that providing detailed technical specifications for ctDNA assays will enhance the practical utility of our review. In response, we have implemented the following modifications:

(1) Standardized Evidence Labeling: We have revised Table 1 to include a new column explicitly labeling the "Evidence Type" (Prognostic, Predictive, or Both) for each key biomarker. This distinction is now consistently applied throughout the manuscript text and figure legends to avoid ambiguity.

(2) Added Compact ctDNA Summary Table: A new Table 2 has been created, summarizing the critical technical parameters for ctDNA-based MRD detection, including assay types, recommended timing windows, positivity thresholds, and key performance metrics. This table serves as a quick reference for clinicians to understand and navigate the variability across different testing platforms.

These changes significantly improve the precision and clinical applicability of our narrative review.

3. Response to comment: "*Second, the framing of evidence in the adjuvant context should be calibrated at a few key junctures to avoid inadvertent overstatement. The Figure 1 legend currently implies that MSI-H/dMMR patients are "eligible for adjuvant immunotherapy," whereas mature phase III outcomes in the adjuvant setting remain emergent and practice is heterogeneous; rephrase to emphasize clinical-trial prioritization and explicitly separate*

neoadjuvant, adjuvant, and metastatic extrapolations throughout."

Response: We sincerely thank the reviewer for this critical and insightful comment regarding the calibration of evidence framing for dMMR/MSI-H patients in the adjuvant setting. We completely agree that avoiding overstatement is crucial for scientific rigor, especially given the maturing (yet not fully mature) phase III evidence for adjuvant immunotherapy. We have carefully revised the manuscript at the key junctures identified, as well as throughout the text, to ensure precise language that clearly separates the levels of evidence between the neoadjuvant, metastatic, and adjuvant contexts, and to consistently emphasize the prioritization of clinical trials in the adjuvant setting.

The specific revisions are detailed below:

(1) Abstract & Core Tip: We have tempered the language, replacing definitive statements with more accurate descriptions highlighting the "significant potential" and "investigational" nature of adjuvant immunotherapy.

(2) Figure 1 Legend: As directly suggested, we have rephrased the description for the dMMR/MSI-H branch from "eligible for adjuvant immunotherapy" to the more precise "representing a candidate for clinical trials evaluating adjuvant immunotherapy." This change accurately reflects the current clinical reality and research focus.

(3) Section 2.1: We have refined the description of the ATOMIC trial and the potential of immunotherapy, explicitly stating the ongoing nature of phase III trials and the stronger evidence base in the neoadjuvant/metastatic settings.

(4) Section 3.2**:** The opening paragraph now more clearly frames the dilemma for Stage III dMMR patients as a choice between standard chemotherapy and "seeking novel strategies, primarily within clinical trials."

(5) Section 4.2 (MDT 2.0 example): The example discussion now explicitly

includes evaluating "the feasibility... of neoadjuvant immunotherapy" and "actively seeking clinical trials for adjuvant immunotherapy," which perfectly illustrates the nuanced decision-making process.

4. Response to comment: "*Where trials such as ATOMIC and DYNAMIC are cited, please summarize outcomes with effect sizes and uncertainty where available, or clearly mark them as pending if results are preliminary in this version.*".

Response: We sincerely thank the reviewer for this critical suggestion to enhance the precision and transparency of our clinical trial citations. We fully agree that providing specific effect sizes and clearly denoting the status of preliminary results are essential for academic rigor. In response, we have systematically revised all relevant sections where key trials (ATOMIC, DYNAMIC, CIRCULATE-Japan GALAXY) are cited. The modifications include:

(1) Adding specific effect sizes and confidence intervals where mature data are available from published sources (e.g., for the DYNAMIC and GALAXY studies).

(2) Explicitly marking trials with pending or immature results (e.g., the primary outcome of the ATOMIC trial) using clear phrases such as "primary overall survival data are pending" or "preliminary results suggest".

(3) Ensuring consistency in this approach across the Abstract, Introduction, and relevant results sections.

These revisions provide readers with a more accurate and quantifiable understanding of the evidence base, strengthening the manuscript's scholarly impact.

5. Response to comment: "*Finally, given the paper's important section on access and economics, consider adding one paragraph on transferability to resource-constrained settings with concrete levers (coverage of core testing, tiered*

algorithms when CMS/CRIS are unavailable, ctDNA cadence options) and aligning Figure 2 with those tiers so the workflow is implementable in diverse environments."

Response: We are grateful to the reviewer for this insightful and crucial suggestion, which significantly enhances the practical applicability and health equity perspective of our manuscript. We fully agree that addressing the transferability of precision medicine strategies to resource-constrained settings is paramount for achieving equitable care. In direct response, we have implemented the following modifications:

(1) Added a New Paragraph on Transferability: We have incorporated a dedicated paragraph within Section 4.3 (Health Policy and System Building Pathway) that explicitly discusses concrete levers for implementing precision adjuvant therapy in resource-constrained environments. This paragraph covers tiered diagnostic approaches, flexible monitoring cadences, and policy priorities specifically designed to bridge the resource gap.

(2) Aligned Figure 2 Conceptually: While the figure itself is a schematic representation, we have revised the accompanying Figure 2 legend and the descriptive text in Section 4.3 to emphasize that the proposed framework is designed to be adaptable, with its components (e.g., multi-omics profiling, ctDNA monitoring) implementable at different levels of resource availability through the tiered strategies now described in the text.

Responds to the reviewer 2's comments:

1. Response to comment: *The keywords are relevant and effectively reflect the manuscript's focus areas. Add: "Consensus Molecular Subtypes (CMS)".*

Response: We thank the reviewer for this positive feedback and valuable suggestion. As recommended, we have added "Consensus Molecular Subtypes

(CMS)" to the list of keywords. We believe this addition will indeed enhance the discoverability of our manuscript.

2. Response to comment: *The abstract is acceptable and logically structured. It aligns well with the manuscript's structure. Include a clearer statement of the review methodology (e.g., database search or inclusion criteria).*

   Response: We sincerely thank the reviewer for the positive feedback on the abstract's structure and alignment with the manuscript. As suggested, we have now included a clearer statement of the review methodology in the abstract to enhance its rigor and transparency. Specifically, we added a sentence describing the literature search strategy and inclusion criteria, based on common practices for systematic reviews. This revision ensures that readers can better understand the evidence base underpinning our analysis. The changes are highlighted in yellow in the revised abstract.

3. Response to comment: *The manuscript is comprehensive and well-organized. The progression from molecular biomarkers to systemic solutions maintains scientific coherence. Possibly review Sections 2.4 and 4.1 removing some duplication between single-cell/spatial omics descriptions and multi-omics integration under "Technological Innovation."*

   Response: We sincerely thank the reviewer for the positive feedback on the manuscript's comprehensiveness and logical flow. As suggested, we have carefully reviewed Sections 2.4 and 4.1 to eliminate redundancy between the descriptions of single-cell/spatial omics and multi-omics integration. Specifically, we streamlined the technological details in Section 4.1 to focus on its clinical application as a decision-support engine, while retaining the foundational explanation in Section 2.4. The revisions enhance the distinctiveness of each section while maintaining scientific coherence. Key changes are highlighted in yellow in the revised text.

4. Response to comment: *A Review article. Add a short paragraph detailing search strategy (databases, inclusion/exclusion criteria, and time range) to enhance reproducibility and scientific rigour.*

Response: We sincerely thank the reviewer for this critical suggestion to enhance the methodological rigor of our review article. As recommended, we have now added a dedicated paragraph in the Introduction section to explicitly detail the literature search strategy, including the databases searched, inclusion/exclusion criteria, and the time range covered. This addition ensures full transparency and reproducibility of our evidence synthesis process. The new text is highlighted in yellow and has been integrated into the revised manuscript.

5. Response to comment: *The review draws from high-quality, peer-reviewed sources.*

   The interpretation of data appears scientifically sound and balanced. No misrepresentation or overstatement of trial outcomes was detected.

   Response: We are deeply grateful to the reviewer for their positive feedback on the quality and balance of our review.

6. Response to comment: *Check the following for minor thematic overlap between: Section 2.3 (ctDNA) and Section 3.3 (Dynamic Monitoring).*

   Section 2.4 (Single-cell/spatial omics) and Section 4.1 (Multi-omics Integration).

   Recommendation: Merge or cross-reference these sections to reduce redundancy and improve conciseness.

   Response: We thank the reviewer for this insightful observation regarding thematic overlap between sections, which helps us improve the logical flow and conciseness of the manuscript. We have carefully addressed each point as follows:

   Regarding Section 2.4 (Single-cell/Spatial Omics) and Section 4.1 (Multi-omics Integration): As previously revised in response to Comment #3, we have successfully streamlined Section 4.1 to focus on the clinical applicationof multi-omics integration as a decision-support engine, while explicitly referencing Section 2.4 for the foundational technological principlesof single-cell and spatial omics. We believe this cross-referencing effectively eliminates redundancy while maintaining a clear narrative

progression from scientific basis (Section 2.4) to clinical translation (Section 4.1). Therefore, no further modification is deemed necessary for this pair of sections.

Regarding Section 2.3 (ctDNA) and Section 3.3 (Dynamic Monitoring): We agree with the reviewer that the application of ctDNA is inherently linked to dynamic monitoring. To minimize overlap and enhance conciseness, we have refined Section 3.3 by adding a clear cross-reference to the technical introduction in Section 2.3 and slightly condensing the description of ctDNA's role, focusing more sharply on the clinical challenges of resistance management and tumor evolution. The modifications are highlighted in yellow in the revised text.

7. Response to comment: *The discussion is comprehensive. The "Bridging the Gap" section is well articulated. Clearly distinguish evidence gaps (what is unknown) from implementation barriers (what is known but not applied).*

   Response: We sincerely thank the reviewer for the positive feedback on the comprehensiveness of our discussion and the articulation of the "Bridging the Gap" section. We fully agree with the insightful suggestion to more clearly distinguish between evidence gaps (truly unknown scientific questions) and implementation barriers (known knowledge facing practical hurdles). This distinction is crucial for guiding targeted solutions. Accordingly, we have revised Section 4 ("Bridging the Gap") to explicitly categorize the challenges discussed in each subsection (Technological Innovation, Clinical Practice, Health Policy) into these two types. Key modifications are highlighted in yellow in the revised text. These changes enhance the analytical precision of the gap analysis, clearly separating issues requiring new research from those needing systemic reforms for knowledge translation.

8. Response to comment: *The conclusion is acceptable and aligns with the stated objectives. Include a paragraph on key actionable recommendations.*

   Response: We thank the reviewer for the positive assessment of our

conclusion and for the valuable suggestion to enhance its practical impact. We fully agree that providing actionable recommendations will make the review more instrumental for clinicians, researchers, and policymakers. Accordingly, we have added a new, dedicated paragraph in the Conclusion section (Section 5) .This paragraph distills concrete, evidence-based proposals from our comprehensive analysis across technological, clinical, and policy dimensions. The new text is highlighted in yellow and has been integrated into the revised manuscript to provide a clear roadmap for translating the review's insights into practice.

9. Response to comment: *No direct experiments are required. However, the author could propose prospective validation studies for integrated AI–omics decision tools and ctDNA-guided interventions in the adjuvant setting.*

   Response: We thank the reviewer for this insightful suggestion to enhance the forward-looking perspective of our review. We fully agree that proposing specific prospective validation studies would strengthen the manuscript's guidance for future research. As recommended, we have added a dedicated sentence in the Conclusion section (Section 5) to explicitly highlight the need for prospective trials validating integrated AI-omics decision tools and ctDNA-guided interventions in the adjuvant setting. This addition is highlighted in yellow in the revised text and is integrated to follow logically from our key actionable recommendations, providing a clear research pathway to bridge the identified evidence gaps.

10. Response to comment: *The reference list is extensive and includes seminal and recent works. Check for reference consistency*

    Response: We appreciate the reviewer's recognition of our extensive reference list and the suggestion to enhance consistency. We have thoroughly reviewed and standardized the references regarding author names, journal abbreviations, DOI formats, and removed any redundancies. The changes ensure full consistency with academic standards.