

Point-to Point Responses to Editorial Office

Dear editor and reviewers:

We sincerely appreciate the thorough analysis and constructive suggestions provided by the reviewers and the editor of our manuscript (Manuscript NO.116028) on our editorial manuscript. These comments are highly valuable for strengthening our arguments and clarifying key points. We have carefully considered each comment and provide a point-by-point response below. Please note that as this is an editorial piece discussing the work of Taskiran et al., our responses focus on refining our interpretation, discussion, and future perspectives based on the available data and the broader scientific context, rather than on presenting new primary data. We have carefully addressed the reviewers' concerns in the following point-by-point responses and revised the manuscript accordingly.

Response to Reviewer 1

This editorial emphasizes the potential significance of non-EDMs (non-enzymatic exon-deletion domains) mutations in DNA polymerase ϵ (POLE) of colorectal cancer (CRC), based on a study reported by Takkirian et al. in the World Journal of Gastroenterology. The study found a higher frequency of POLE mutations in the Turkish CRC population, mainly driven by the frameshift mutation p.V1446fs*3, whose pathogenicity remains to be clarified. It is notable that non-EDMs co-occur significantly with key gene mutations such as MLH3, MSH3, KRAS, PIK3CA, and BRAF, suggesting that DNA repair defects may synergize with the activation of oncogenic pathways. Although POLE mutation tumors are mostly of non-MSI-H phenotype, their high mutation load may still confer their immunotherapy sensitivity. Therefore, the authors call for functional experiments and multicenter studies to verify the biological and clinical significance of non-EDMs, and recommend including comprehensive POLE sequencing covering non-EDM regions in the molecular classification of CRC to optimize individualized treatment strategies. The overall language of this article is fluent, the structure is complete, and the logic is clear. It has certain reference value and enlightenment significance for the related research field. I believe that after making

appropriate modifications and improvements to some details, this article can be accepted.

Comment 1: The first occurrence of abbreviations should have their full definitions, for example, dMMR, although the full name is written when it appears for the second time.

Response: Thank you for pointing out this important detail. We fully agree with your comment. In the revised manuscript, we have thoroughly reviewed all abbreviations and ensured that each one is explicitly provided with its full name upon its first occurrence. This has been revised according to your suggestion.

Comment 2: Adding line numbers/page numbers would make it more convenient for reference.

Response: Thank you for this helpful suggestion. We fully agree that adding line/page numbers would greatly facilitate reviewing and referencing. In the revised manuscript, we have added continuous line numbers (or page numbers) throughout the text to enhance its readability and ease of reference.

Comment 3: The references can be supplemented with some from 2025 to enhance the timeliness and completeness of the literature review section.

Response: Thank you for this valuable suggestion. We fully agree that supplementing the references with recent publications from 2025 will significantly enhance the timeliness and completeness of the literature review section. In the revised manuscript, we have searched for and incorporated relevant key literature published in 2025 to ensure the citations are up-to-date.

Comment 4: The key words are quite numerous. They can be appropriately reduced to 5.

Response: Thank you for this careful observation. We agree that the initial number of keywords was excessive. Following your advice, we have reduced the keywords to the 5 most representative and searchable core terms.

Comment 5: As an editorial, I think the manuscript is somewhat lengthy, and a moderate

reduction in length is recommended.

Response: Thank you for this constructive feedback. We understand and agree with your perspective that an editorial should be more concise. In the revised version, we have thoroughly reviewed the entire manuscript. We have removed redundant or non-essential discussions, consolidated some paragraphs, and refined the language throughout. These revisions have made the manuscript more concise and focused while preserving the integrity of its core arguments.

Response to Reviewer 2

This manuscript by Jia-Ju Xu et al. provides a comprehensive editorial on the significance of non-exonuclease domain mutations (non-EDMs) in DNA polymerase epsilon (POLE) in colorectal cancer (CRC), building on the recent study by Taskiran et al. The authors highlight the potential clinical implications of these mutations, emphasizing the need for functional validation and integration into molecular subtyping frameworks. While the manuscript offers valuable insights and a forward-looking perspective, several critical issues need to be addressed to strengthen its scientific rigor and clarity.

Comment 1: The manuscript emphasizes the high frequency of the p.V1446fs*3 variant in the Turkish cohort but lacks detailed functional validation. Given the potential population-specific effects, the authors should provide in vitro or in vivo evidence to elucidate the functional consequences of this variant on POLE activity and tumor biology.

Response: We agree with the reviewer's assessment regarding the critical need for functional validation. As an editorial, our role is to interpret findings and highlight future research directions rather than to generate primary data. To address this, we have significantly strengthened the manuscript by explicitly outlining the necessary experimental path forward. In the revised text, we now propose specific in vitro and in vivo approaches (e.g., CRISPR-edited models) to characterize p.V1446fs*3. This addition transforms the commentary from merely noting a gap into actively defining the research agenda to fill it, thereby enhancing the editorial's value in guiding future studies.

Comment 2: The high prevalence of non-EDMs observed in the Turkish cohort may not be representative of other populations. The authors should discuss the limitations of extrapolating these findings to diverse ethnic groups and propose strategies for multi-center validation studies to assess the generalizability of their conclusions.

Response: We agree with the reviewer's point on the importance of generalizability. We have reframed this potential limitation as a key research question in its own right. In the revised "Potential and Challenges" section, we now explicitly state that the population-specific high frequency of p.V1446fs*3 necessitates and motivates large-scale, multi-center studies across diverse cohorts. This revision effectively positions the initial finding as a catalyst for broader validation work, ensuring our conclusions are appropriately cautious and forward-looking.

Comment 3: The manuscript mentions that most POLE-mutant tumors are MSI-Low or MSS but speculates on their potential hypermutator phenotype. This inconsistency needs clarification. The authors should provide data on tumor mutational burden (TMB) or reference studies that directly link non-EDMs to TMB levels to support their claims regarding immunotherapy responsiveness.

Response: We thank the reviewer for this comment, which allowed us to clarify a nuanced but crucial point. We have revised the manuscript to explicitly distinguish between MSI status and TMB as independent biomarkers. Our argument is that some POLE non-EDMs could confer immunotherapy sensitivity in MSS/MSI-L tumors not through MSI-H, but by elevating TMB—a hypothesis supported by cited literature [24,25]. We now clearly state this distinction and have tempered claims regarding the p.V1446fs*3 variant specifically, noting its observed context does not suggest a classic hypermutator phenotype. This clarification resolves the apparent inconsistency and sharpens our discussion on immunotherapy potential.

Comment 4: The manuscript advocates for comprehensive POLE sequencing but does not address the challenges of standardized detection. The authors should outline specific

recommendations for high-quality next-generation sequencing (NGS) protocols and bioinformatics pipelines to ensure accurate identification of POLE mutations in clinical settings.

Response: We agree with the reviewer that practical recommendations are essential for clinical translation. We have expanded the “Potential and Challenges” section to include concrete, actionable recommendations. Specifically, we now advise on the use of comprehensive NGS panels, adequate coverage depth, and bioinformatic pipelines calibrated for robust indel detection (crucial for variants like p.V1446fs*3), citing relevant technical guidelines [39-41]. This addition bridges the gap between conceptual advocacy and practical implementation, significantly strengthening the translational relevance of our editorial.

Comment 5: The manuscript suggests that POLE non-EDMs could guide personalized treatment strategies, including immunotherapy. However, the clinical evidence supporting this assertion is limited. The authors should temper their conclusions and emphasize the need for prospective clinical trials to validate the predictive value of non-EDMs for treatment outcomes.

Response: We appreciate the reviewer’s caution and have rigorously tempered our language regarding immediate clinical application. The revised text now positions POLE non-EDMs as a “potential” biomarker class and underscores that their predictive value remains “hypothetical” without prospective validation. We have strengthened the conclusion to make an unequivocal call for “prospective clinical trials that stratify patients based on specific, functionally validated POLE non-EDMs.” This revision ensures our tone is appropriately balanced, framing current evidence as a foundation for future research rather than a definitive guide for current practice.

Comment 6: The discussion of co-mutations with MLH3, MSH3, KRAS, PIK3CA, and BRAF is complex and lacks clarity. The authors should provide a more structured analysis of how these co-mutations interact with POLE non-EDMs, and their combined impact on tumor

behavior and treatment response. Additionally, the manuscript should include a summary table of key co-mutations and their clinical relevance.

Response: We thank the reviewer for this constructive suggestion. To enhance clarity and synthesis, we have: (1) added a new Table 1 summarizing the key co-mutated genes, their functional categories, and potential clinical relevance; and (2) restructured the corresponding narrative to sequentially discuss the implications of co-mutations in DNA repair genes, oncogenic pathways, and tumor suppressors. This new structure culminates in an integrated model of how these interactions may collectively drive POLE-mutant CRC. These revisions provide the structured analysis requested and greatly improve the readability and impact of this section.

We believe that these revisions have substantially strengthened the manuscript, and we are grateful for the reviewers' time and expertise. We hope the revised version is now suitable for publication.