Santiago, September 25th, 2023

Jin-Lei Wang,
Company Editor-in-Chief
Editorial Office
World Journal of Clinical Oncology

Dear Professor Wang,

Thanks for your invitation to contribute to the World Journal of Clinical Oncology. We are pleased to provide you with a revised version of our manuscript entitled **"Classification of patients with metastatic colorectal cancer into consensus molecular subtypes into real-world: A pilot study",** to be further considered for publication at the journal.

In the revised version of the manuscript, in agreement with Reviewer's comments, we have aimed to make a new analysis of the clinical, RT-PCR, and next-generation sequencing results. This was done to facilitate a comprehensive analysis of the findings and to prevent any potential confusion in their interpretation. Furthermore, at the request of the Reviewers, we have included the overall survival data for the n=26 patients studied. Additionally, detailed results of the molecular biology techniques employed for each patient, as well as information on the primers used in the RT-PCR experiments, have been added. In line with the Reviewers' recommendations, a new analysis has been incorporated to provide a deeper understanding of the implications of our results. Figure 1 has been completely revised, and Figures 2A-B have been added. Furthermore, the tables have undergone a complete overhaul, with the comprehensive summary of our experimental results now presented in Tables 1, 2, and 3.

A detailed, itemized response to the comments of the Reviewers is provided in a separate file. This article has not been submitted to another journal. All the authors are in agreement with its content and there are no potential conflicts of interests.

I look forward to hearing from you at your earliest convenience.

Sincerely yours,

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Detailed, itemized response to Reviewers 'comments.

Reviewer #1:

1- The methods for RT-PCR experiments, CMS categories identification and data analysis, described in this manuscript doesn't present in adequate detail. What is the information for those RT-PCR primers which should be provided in a supplementary table?

Response: We thank the Reviewer for the comment. The methods for the RT-PCR experiments are now adequately detailed in the main text within the "Methods" section and are highlighted in yellow in the text. Information regarding the primers used for the RT-PCR experiments is provided in Table 1 (new table), which has been added as per the Reviewer's request. "Furthermore, 7 new references [9-15] have been added, detailing the primers utilized in our RT-PCR experiments, which are highlighted in yellow within the text.

2 - The original dataset of all those gene mutations or expression for each patient better be shown in a supplementary table according to the detailed criteria to classify their CMS categories.

Response: We thank the Reviewer for the comment. Based on the Reviewer's comments, a new analysis of patients was conducted, excluding "non-categorically" CMS (Consensus Molecular Subtypes) to prevent confusion in result interpretation. Consequently, a total of n=26 patients were ultimately analyzed using the three molecular biology techniques. Table 2 has been added to provide detailed clinical data, overall survival and the expression of β-catenin, c-MYC, and TGF-β measured by RT-PCR for each patient, along with their molecular subtype (CMS) assigned by the Tumor Board consensus. Furthermore, in response to the Reviewer's comments, Table 3 has been included, presenting the results obtained from the application of the TumorSec panel for massive genomic sequencing in each of the patients.

3- Figure 1 need be more clarified and better replaced with a spot diagram.

Response: We thank the Reviewer for the comment. The information that was previously included in Figure 1 has been replaced with Table 2, providing a detailed account of the

expression levels of the genes β-catenin, c-MYC, and TGF-β for each of the patients under study. This change was made to ensure precise and comprehensive information regarding the expression of these three genes.

4- To provide a comprehensive overview of the mutations in those 25 genes with their 25-gene TumorSec panel, a clarified and classified table is much more informative including each genetic status within each group of patients rather than a simple figure with the proportion of patients harboring mutation in Figure 2.

Response: We thank the Reviewer for the comment. In response to the Reviewer's comment, Table 3 has been incorporated, illustrating the outcomes of Next-Generation Sequencing (NGS), specifically TumorSec, for each of the 26 patients included in the final analysis of the study. This data now supersedes the content originally presented in Figure 2 (included in the initial submission of our manuscript).

5- The results in Figure 3 is confused and too inadequate to describe those four identified CMS system together with 6 unclassifiable patients. Based on their results, the authors addressed that among those 24 identifiable patients, the remaining 8 patients (33%) were classified as non-categorical but probable for a CMS, but how they further determined those are CMS4 patients? Need more clear statement and discussion.

Response: We thank the Reviewer for the comment. We acknowledge that the explanation of our results in the initial version of our manuscript was indeed unclear. In response to the Reviewer's comments, we reevaluated both our results and the categorization of patients into different CMS and eliminated the "non-categorically" classification to prevent any confusing interpretation of these findings. Consequently, the final analysis of our results encompassed a total of n=26 patients (Tables 2 and 3). Out of these n=26 patients, a proper categorization was achievable for n=24 patients (all in a categorical manner), while n=2 patients could not be classified into any of the 4 consensus molecular subtypes by the Tumor Board. By presenting our results in this manner, we aim to mitigate any potential for misinterpretation. This clarification has been incorporated into both the abstract and the results section of our manuscript.

6 - The same correction need be done in the Abstract where they declared that “Thirty patients ….. in this study. Among them, 20% (n=6), 10% (n=3), 23% (n=7), and 27% (n=8) were classified as CMS1, CMS2, CMS3, and CMS4, respectively……Notably, 67% of cases were determined to belong to categorical CMSs, while the remaining 33% belonged to non-categorical CMSs”.

Response: We thank the Reviewer for the comment. The same correction as explained in the previous response (response number 5) was applied to the abstract, and this correction was highlighted in yellow within the text.

7- They since have obtained the gene expression- /or mutation-based subtype signatures for those genes, I am very interested in that they might also be able to find important associations between the CMS groups and clinical variables/ and differences in prognosis. There would be more scientific significance in their findings if the results could confirm that the clinical relevance of the intrinsic biological processes implicated in each CMS.

Response: We thank the Reviewer for the comment. While the primary objective of this project was not - initially - to report on overall survival (OS) of the studied patients, this critical information will be included in response to the request made by the Reviewer.

We have determined the OS of the studied patients. Out of the 26 patients included on the final analysis, an OS of 28 months was observed (new Figure 2A). Among the 24 patients for whom it was possible to assign a CMS, the following OS values were obtained: CMS1: 11.5 months. CMS2: 20 months, CMS3: 30 months and CMS4: 45 months (new Figure 2B). The statistical analysis of OS using the logrank test yielded a p-value of 0.0968, which is not statistically significant. This implies that, although there was a numerical difference in the OS among the studied CMS groups, this difference did not reach statistical significance, and does not allow for concluding analyses regarding whether the biological characteristics of the different CMS have an impact on OS. At the Reviewer's request, these results have been integrated into the primary text within the "Results" section and have been included in the abstract, with the new text highlighted in yellow within the manuscript.

8- What are the new findings or novelty of this study? Or brought any new concepts in this study proposes? The author might describe more in the Discussion section.

Response: **We thank the Reviewer for the comment. The main novelty of this study is the feasibility of classifying patients with metastatic colorectal cancer into one of the four consensus molecular subtypes using a protocol involving simple molecular biology techniques (RT-PCR, and NGS), based on the molecular characteristics defined by Guinney et al. in 2015. To date, there is no universally accepted method within the scientific and clinical community for categorizing patients into different CMS categories, making this protocol a potential avenue for shedding light on the optimal approach to such classification. This idea was added to "Discussion" section, according the Reviewer comment, and highlighted in yellow in the text.**

Company editor-in-chief:

1 - I have reviewed the Peer-Review Report, full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Clinical Oncology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.

Response: All Figures and Tables was prepared using PowerPoint in an editable format.

2 - In order to respect and protect the author's intellectual property rights and prevent others from misappropriating figures without the author's authorization or abusing figures without indicating the source, we will indicate the author's copyright for figures originally generated by the author, and if the author has used a figure published elsewhere or that is copyrighted, the author needs to be authorized by the previous publisher or the copyright holder and/or indicate the reference source and copyrights. Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper).

Response: **All Figures and Tables are original and generated de novo by the author for this paper.**

3 - If the picture is ‘original’, the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2023.

Response: **The copyright information was added according the instruction on each Figure and Table on Power Point file.**

4 - Authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content.

Response: The Table was corrected according the instructions of Company editor-in-chief.

5 - Please upload the approved grant application form(s) or funding agency copy of any approval document(s).

Response: This study was funded by Agencia Nacional de Investigación y Desarrollo (ANID), FONIS grant number SA20I0059. The approved grant application form was uploaded.