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Dear Ruo-Yu Ma
Science Editor, Editorial Office
World Journal of Gastroenterology

We wish to re-submit the manuscript titled "Clinical assessment and identification of immuno-oncology markers concerning the 19-gene based risk classifier (TCA19) in stage IV colorectal cancer". The manuscript ID is 43477.

We appreciate you and the reviewers for their thoughtful suggestions and insights. The manuscript has benefited from these insightful suggestions. I look forward to working with you and the reviewer to move this manuscript closer to publication in *World Journal of Gastroenterology*

The manuscript has been rechecked and the necessary changes have been made in accordance with the reviewer's suggestions. The point-by-point responses to all the reviewer's comments have been prepared and are attached herewith. The changes in the revised manuscript are marked in red.

Thank you for your consideration. We look forward to hearing from you.

Responses to the Reviewers' Comments:

Reviewer #1

1. *"Samples in this study were limited. Results were from RT-qPCR, which was not stable for repeating."*

Response:

We thank the reviewer for this comment. In accordance with the reviewer's comment, we commented the limited samples in the discussion section. According to the reviewer's suggestion, we added 27 samples for the RT-qPCR and added the result with supplementary table.

(Revised manuscript, page 12 lines 1-5 and supplementary table 5).

"In additional analysis of 27 patients (separate from the 60 study cohort and the 10 validation set), SLAMF7 expression in 7 patients was lower in tumor tissue than in normal tissue (mean $2^{-\Delta\Delta Ct}$ value: 20.21) and TREM1 expression in 18 was higher in tumor tissue than in normal tissue (mean $2^{-\Delta\Delta Ct}$ value: 37.21) (Supplementary table 5)."

Reviewer #2

1. ***"****This manuscript explores the possible use of TCA19 in stage IV CRC. SLAMF7 express in the CRC cells is low, and TREM1 is high. Suggest : Part of the experimental method needs to be described in more detail."*

We thank the reviewer for this comment. According to the reviewer's suggestion, we added figure to explain the algorithm of the study and additionally described RT-qPCR method in more detail.

(Revised manuscript, page 4 lines 19 and page 5 lines 18-22).

"(Supplementary figure 1)"

"in which ΔCt values between one of the TCA 19 genes and the GAPDH control [$\Delta Ct = (Ct)_{TCA19\ genes} - (Ct)_{GAPDH}$].[18] ΔΔCt was defined as a difference in the ΔCt values between a

normal tissue and a tumor tissue of the same patient [ΔΔCt = (ΔCt)normal – (ΔCt)tumor] as previously reported.[18] A $2^{-\Delta\Delta Ct}$ value over 1-fold indicates upregulation of the tested TCA 19 gene in a tumor tissue compared with a normal counterpart of the same patient."

Reviewer #3

1. *"With regard to the 10 patients enrolled for validation, the authors should explain why they selected 10 patients and also provide the baseline characteristics Discussion "in our cohort of 60 patient with stage IV CRC……for targeted regimens compared with the 5-FU regimen": it is necessary to explain that the groups consist of a number of different patients (8 vs 4). Furthermore, the authors should comment that the results were observed in small groups of patients."*

Response:

We thank the reviewer for this comment. In accordance with the reviewer's comment, we commented the limited samples in the discussion section. According to the reviewer's suggestion, we added 27 samples for the RT-qPCR and added the result with supplementary table.

(Revised manuscript, page 12 lines 1-5 and page 15 lines 11-13).

"In additional analysis of 27 patients (separate from the 60 study cohort and the 10 validation set), SLAMF7 expression in 7 patients was lower in tumor tissue than in normal tissue (mean $2^{-\Delta\Delta Ct}$ value: 20.21) and TREM1 expression in 18 was higher in tumor tissue than in normal tissue (mean $2^{-\Delta\Delta Ct}$ value: 37.21) (Supplementary table 5)."

"Our study had some limitations, including the small number of patients with the limited use of targeted regimens to assess a clinical implication of the TCA19 risk score and to validate SLAMF7 and TREM1 as suitable candidate."

2. *"In the title there is the term "validation" and also in the aim of the abstract the authors write*

"validation" On the other hand in the "conclusion session" they underline ." TCA 19 may provide prognostic information… "Further mechanistic and functional studies with large cohorts are now required to confirm….." . The term "validation" should be replaced in the title and in the abstract."

Response:

We acknowledge that the reviewer's concerns are correct. According to the reviewer's comment, we changed the term "validation and validate" in the title and the aim.

(Revised manuscript, page 1 lines 3 and page 2 lines 3).

"**Clinical assessment and identification of immuno-oncology markers concerning the 19-gene based risk classifier (TCA19) in stage IV colorectal cancer**"

"Our aim was to assess clinical implication of TCA19 in patients with stage IV CRC, and to identify TCA19 genes with involvement in immune-oncology."

Again, we sincerely appreciated your pertinent comments enabling our study to be more informative.

Yours sincerely,

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