

ANSWERING REVIEWERS



Dear Editor,

Enclosed, please find our edited manuscript in Word format (file name: 2429-review.doc).

Title: The Correlation between HER-2 Protein Expression and Colorectal Cancer

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The manuscript has been improved according to the suggestions of reviewers:

To Reviewer 71764:

1. This article represents a novel meta-analysis of Her-2 expression in colorectal adenocarcinoma. Explicit explanation of all of the CASP questions are not necessary, and could be omitted

Response: Thank you for the suggestion. We have omitted the CASP score.

2. Logical meta-analysis protocols have been followed, although some concern exists regarding the virtual unanimity of the results, as the authors themselves admit that this is a controversial area. You do not appear to have included references 11 & 12 within the statistical analysis.

Response: Thank you for the suggestion. Based on the inclusion and exclusion criteria, we have excluded references 11 & 12. Reference 11 did not have relative data, and reference 12 was not a case-control study.

3. Indeed, you state that 30 publications are used for the analysis, but only 14 are included in the Forest plots. This disparity is not commented upon in the text.

Response: Thank you for the suggestion. There are 5 indexes in total, and several differences existed in each study. Therefore, the analysis for each index was conducted using a subset of the studies.

4. In study selection, there are 2 consecutive sentences stating that differing numbers of articles were excluded due to data integrity, 41 and 75, respectively. Is there a different reason behind exclusion of either group, or could this figure be combined? This is not clear.

Response: Thank you for the suggestion. The inclusion and exclusion criteria were stated in the methods: **“(1) research type: case-control; (2) research subjects: patients with CRC (the case group) and healthy controls (the control group); and (3) the chosen studies provided complete data such as the number of cases, country, race, age, gender, ethnicity, pathological type, detection method, and the expression of the HER-2 protein.”** We have revised figure 1 according to your comment.

5. Statistical analysis seems sound within the selected papers. The forest plots in figure 3 are difficult to interpret as no reference has been made as to what represents either a case or a control.

Response: Thank you for the suggestion. We have added references to support the results of the forest plots in the Introduction as follows: **“In CRC, HER-2 overexpression has been correlated with advanced tumor stage”**

6. In their current state they suggest that Her-2 expression is favoured in controls, which is against what has

been concluded from the data.

Response: Thank you for the suggestion. First, we would like to apologize for this mistake, which we have already fixed. If the horizontal lines lie to the right of the vertical line, it indicates that the experimental factors are effective.

7. Comments regarding publication bias are difficult to follow. If there is publication bias in CRC patients as a whole as stated in the paper, then why has ongoing analysis been performed at all?

Response: Thank you for the suggestion. Even if the specific retrieval strategy and means were taken into account in the study (such as contact with the original researchers), the publications would still exist, and we cannot include all of the relative studies. Furthermore, we used a case-control experimental design to obtain reliable results.

8. It suggests a wider inclusion of papers with negative results is needed, which also fits with my earlier suggestion that the range of papers included seems rather biased in favour of positive results.

Response: Thank you for the suggestion. We added an extra experiment to provide a more specific method and more reliable results.

9. The discussion is rather brief, and could be extended to comment upon the likely role for herceptin in adjuvant treatment of colorectal cancer.

Response: Thank you for the suggestion. We modified it to: **“Recently, Herceptin, a humanized monoclonal antibody against HER-2, has been shown to have clear therapeutic effects in strongly HER-2/neu-positive breast carcinoma patients, especially when combined with other chemotherapeutic drugs. Thus, further studies on the effect of HER-2 on CRC will demonstrate if Herceptin can also be used as a target for CRC therapy.”**

10. The conclusion that Her-2 expression is correlated with advanced disease is not justified, as there is no significant difference in expression when assessed by TNM stage, but just Dukes stage - I am unsure how this disparity exists as the entities are very similar. The reasons for this disparity need to be commented upon.

Response: Thank you for the suggestion. We added an extra experiment to provide a more specific method and more reliable results.

11. Do you for instance only mean the 'T' component of the TNM staging system?

Response: Thank you for the suggestion. We evaluated the T (tumor invasive depth) stage and N (lymph node metastasis) grade. However, because the sample size was limited, we could not evaluate the M (distant metastasis) grade.

12. IHC is notoriously difficult to get right in new settings, and standardisation is not agreed.

Response: Thank you for the suggestion. The primary method used in our country to detect the HER2 protein is IHC. Indeed, there are several disadvantages to IHC, as we state in the Limitations: **“the HER-2 protein was detected using IHC, whose results could be influenced by various detection approaches and subjective criteria. However, we could not find a more suitable technique that is currently in use in China. In this regard, further study with more accurate detection methods could be warranted.”**

13. Additionally, what criteria for positivity of HER-2 expression were used in the publications - strong staining, or just some staining. Is comment made as to where within the tumour the staining is present? You mention using FISH, but there is no reference or reason for suggesting this - please expand.

Response: Thank you for the suggestion. We performed an extra experiment to provide a more specific method and more reliable results.

14. How do the authors account for the disparity in significance between 3 and 5-year DFS? Is Her-2 a marker of likely recurrence, and if so, why is this not observed at 5 years?

Response: Thank you for the suggestion. We sincerely wanted specific results, but there were not enough original data. Therefore, an experiment was conducted, and the results showed that the expression of the HER-2 protein in CRC was correlated with the Dukes' stage, the depth of invasion and LNM but not with gender, age, tumor location, tumor size, or degree of tumor differentiation. The HER-2-positive patients had lower 3-year OS and 5-year OS rates when compared with HER-2-negative patients, but there was no significant difference. However, there was a statistically significant difference between the 3-year DFS and 5-year DFS rates of HER-2-positive and HER-2-negative patients.

15. Overall, the article needs polishing in terms of its written English as some phrases are difficult to understand which impacts on the readability of the paper.

Response: Thank you for the suggestion. We have sent this manuscript to a language and editing service to improve the language.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.