Author's response to reviews

Title: Association between ERCC1 and TS expression levels and disease free survival in colorectal cancer patients in response to oxaliplatin and fluorouracil (5-FU) adjuvant chemotherapy

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Author's response to reviews: see over
Dear Dr. Tonilynn Manibo,

Manuscript ID 7583331891188427 entitled "Association between ERCC1 and TS mRNA levels and disease free survival in colorectal cancer patients receiving oxaliplatin and fluorouracil (5-FU) adjuvant chemotherapy" has been revised. The authors would like to thank Reviewers for their careful review of our manuscript and providing us with their comments and suggestion to improve the quality of the manuscript. The point-by-point responses to these comments are listed below. We would like to re-submit this revised manuscript to “bmc gastroenterology” and hope it is acceptable for publication in the journal. Please do not hesitate to contact us with additional questions.

**Our response to Reviewers' Comments:**

**Reviewer: 1**

**Major comment:** The major flaw is the methodology. The authors seem to be looking at too many things; is the study trying to test a new regime (chemo 2) with higher oxaliplatin dose, to look for ERCC1 and TS expression level in DFS or to look at the correlation between these markers and response to oxaliplatin? If the last aim is the major one, I don't think this was clearly demonstrated at all. A comparison would have to be made between two matched groups using a standardized oxaliplatin based regimen and then comparing the expression levels between the two groups.

**Response:** Thanks for your kind suggestion. The aim of this study was to explore the association of ERCC1 and TS mRNA levels with the disease free survival (DFS) in Chinese colorectal cancer patients receiving oxaliplatin and 5-FU based adjuvant chemotherapy. The original manuscript has many unappropriated analysis. The DFS curves according to the mRNA expression levels of ERCC1 and TS have instead the DFS curves according to chemotherapy regimens.

**Minor comment:** The conclusion topic was inserted wrongly, should only be before the last concluding paragraph.

**Response:** Thanks for your careful review. We have revised it according to your suggestion.

**Reviewer: 2**

**Comment 1:** Please check your citations carefully. Several of the ones provided appear inappropriate (for example – citation 1 refers to a paper on renal cell cancer, while citation 2 deals only with metastatic colon cancer and is not reflective of the overall survival. The leading statement – colorectal cancer is highly aggressive is also something of an overstatement as outcomes depend on the stage and early stage colon cancer has excellent outcomes.

**Response:** Thanks for you kind suggestion, we have revised the introduction and
checked all the citation in the whole manuscript.

**Comment 2:** The manuscript would benefit from closer attention to language and grammar. Several sentences are inappropriately constructed – for example, Page 3, end of first paragraph – “There is, however, no predictive factor of response....” is poorly constructed and erroneous in structure and grammar.

**Response:** Thanks for your kind suggestion. We have checked the whole manuscript and revised them with the help of colleagues who have the better English.

**Comment 3:** A total of 112 patients were included in the present study. Please describe the total patient population with CRC being seen / who underwent surgery during the same time period. What is the generalizability of this cohort? Is the sample representative?

**Response:** Thanks for your kind suggestion. The patients in this study represent the Chinese colorectal cancer at stage II-III. All the patients were treated with chemotherapy after surgery.

**Comment 4:** Was any power calculation performed a priori? How was the necessary cohort size estimated?

**Response:** Thanks for your kind suggestion. The sample size of this study was 112 and the statistical analysis was all performed based on the suggestion of expert.

**Comment 5:** Patients were eligible for inclusion if they had stage II or stage III cancer. Yet the results state that tumor metastasis was identified in forty patients – please clarify.

**Response:** Thanks for your kind suggestion. We have clarified this data. There were forty four patients which occurred tumor metastasis up to January 2014.

**Comment 6:** Under the different chemotherapy regimens – please rephrase the ‘normal intravenous injection’ to specifically refer to the combination described in the methods. The term ‘normal intravenous injection’ is not appropriate. Please do not use phrases like chemo 1, chemo 2, etc. – it would be preferred to refer to the chemotherapy regimen itself.

**Response:** Thanks for your kind suggestion. We have revised the manuscript according to your suggestion. The chemo1, chemo 2, etc. have been instead the chemotherapy regimen 1, chemotherapy regimen 2, etc.

**Comment 7:** How was TS and ERCC1 expression level quantified? As a continuous variable? Prior studies have utilized thresholds which may be biologically relevant in classifying patients as high or low expression. Did you attempt this analysis and were signals identified there suggesting a possible threshold effect?

**Response:** Thanks for your kind suggestion. We have revised the manuscript according to your suggestion. Student's t test and the Wilcoxon rank sum test were used for continuous variables, and Pearson's X2 test and Fisher's exact test were used.
for categorical variables.

**Comment 8:** Please use standard terminology – Page 7 – what is tubinva, nervinva?

**Response:** Thanks for your kind suggestion. This is our mistake. We have revised tubinva to vascular invasion and nervinva to nerve invasion.

**Comment 9:** It is not clear at all how variables were modeled in the univariate or multivariate analysis and what the reference groups are. For example, stage – what does the hazard ratio represent? Stage III vs. stage II? How about duration? Dose of chemotherapy? Text on page 7 refers to it as univariate analysis while the title of the table is listed as multivariate analysis.

**Response:** Thanks for your careful review. In the model, DFS was the dependent variable; other factors were the independent variable. In multivariate analysis, stepwise regression was used to variable selection.

**Comment 10:** A big limitation of this manuscript is the small number of patients within each chemotherapy regimen category. This substantially limits the power to identify an association between TS or ERCC1 expression and DFS.

**Response:** Thanks for your careful review. This is the limitation of this study and we have elaborated it in the discussion.

**Comment 11:** In their discussion – the authors stage that because of HR of 0.818 for TS expression – it may still be predictive of response to chemotherapy. This is not an appropriate claim to make for non-statistically significant results. The univariate p-values are actually close to 1.0 suggesting no effect at all. The discussion should be focused on why they did not identify an effect while previous studies in GI or Gyn cancers may have identified an effect.

**Response:** Thanks for your kind suggestion. We have revised the discussion according to your suggestion.

**Minor points:**

**Comment 1:** The title should preferably be free of abbreviations as well. Consider revising so it is appropriate.

**Response:** Thanks for your kind suggestion. We have revised the title to “Association between ERCC1 and TS mRNA levels and disease free survival in colorectal cancer patients receiving oxaliplatin and fluorouracil (5-FU) adjuvant chemotherapy”

**Comment 2:** There is some literature of ERCC1 and response to cisplatin in ovarian cancers. Consider included that in the introduction.

**Response:** Thanks for your kind suggestion. We have revised the introduction according to your suggestion

**Comment 3** In the results, please quantify quality of mRNA in preserved specimens,
and if any purification was needed.

**Response:** Thanks for you kind suggestion. The mRNA of TS and ERCC1 was relative to the housekeeping gene β-actin. The mRNA has been purified in this study.

We appreciate very much for your time in editing our manuscript and the referees for their valuable suggestions and comments. We are looking forward to hearing from your final decision when it is made.

With kindest regards,
Sheng Li