Author's response to reviews

Title: Class III beta-tubulin is a predictive marker for taxane-based chemotherapy in recurrent and metastatic gastric cancer

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Author's response to reviews: see over
Dear Sir,

Please consider for publication of our manuscript “Class III β-tubulin is a predictive marker for taxane-based chemotherapy in recurrent and metastatic gastric cancer” as a clinical research paper (The subject category: clinical research).

Class III β-tubulin (TUBB3) has been shown to play a role in chemotherapy resistance in various cancer types. However, the role of TUBB3 in gastric cancer has not been widely investigated, although it is important in the treatment of gastric cancer to predict chemosensitivity with the goal of improving the response rate and overall survival (OS), and preventing unnecessary side effects and useless treatments. In this study, we analyzed the significance of TUBB3 and ERCC1 in recurrent and metastatic gastric cancer patients receiving taxane-based first-line palliative chemotherapy.

The manuscript submitted is original, is not under consideration or has not been previously published and its content has not been anticipated by any previous publication. All authors have reviewed the manuscript, agree with its contents, and approve of its submission of “BMC Cancer” for publication consideration.

We would be grateful if the manuscript could be reviewed and considered for publication in BMC Cancer.

Sincerely yours,

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Response to reviewer's report (reviewer: Yihong Wang)

1. The clinical follow up time for 13.1 months was relatively short for a good evaluation of PFS.

   We apologize for the error. This number refers to the duration of OS instead of the follow-up time. We have revised the manuscript as follows:
   The median follow-up duration (from the first visit to death or the date of last follow-up) was 23.7 months (range, 4.9-75.4 months).

2. Among 146 patients in this study, 90 of them were initially presented as metastatic disease which deemed unresectable. However, 56 of the patients in this study had previous resectable disease with prior surgery resection. Mixing the two groups for PFS and OS may not be appropriate for analyzing and draw appropriate result.

   We agree. Mixing the two groups to determine the PFS and OS may not be appropriate. However, palliative chemotherapy was administered to patients with recurrent or initially metastatic gastric cancer. In most studies evaluating palliative chemotherapy for gastric cancer, chemotherapy was administered to these two groups; therefore, we included these two groups in our PFS and OS analyses. We have included your comments as a limitation of this study in the Discussion section as follows: Second, this study included a somewhat heterogeneous patient population. Among 146 patients, 90 initially presented with metastatic disease, whereas 56 had recurrent disease after curative resection.

3. The chemotherapy duration and schedule were also different as 92 received full dose, but 54 did not. Some could argue that during the 13.1 month follow up, some difference may be the chemotherapy does difference.

   We agree. We have revised the manuscript accordingly as follows:
   → Despite demonstrating the predictive significance of TUBB3 expression, the present study has several potential limitations. First, it was a retrospective analysis from a single institution. Therefore, the chemotherapy dose and schedule might be different from patient to patient according to individual patient organ function, tolerability, and toxicity profiles.
4. Immunohistochemistry analysis for tubulin III has been used in multiple papers with different cut off values, mostly use 50% of tumor population with positive staining or “median” staining in the study series as the cut off, but current paper used H-score with 4-6 considered high. This core system make it difficult to compare to the published papers on tubulin III. The authors did not state whether the immunohistochemistry staining is performed mostly on the core biopsy or resection sample.

   In this study, we considered both the percentage of tumors showing staining as well as the staining intensity; that is, we used the H-score. We also used the median value of the H-score for both TUBB3 and ERCC1 (median value = 4). Only one study has assessed TUBB3 staining in gastric cancer (Urano et al., Int J Oncol 2006;28:375-381). Most published reports on TUBB3 determined the percentage of tumors showing staining from patients with lung, ovarian, or breast cancer. However, a recent study of the correlation between TUBB3 and locally advanced head and neck cancer used the H-score (Koh et al., Ann Oncol 2009;20:1414-1419), as did a study of the correlation between ERCC1 and non-small cell lung cancer (Lee et al., Lung Cancer 2009;65:377-382). The latter study used the median value as the cut-off. The median value of all H-scores was chosen a priori as the cut-off value for identifying proteins with high and low levels of expression.

   The use of core biopsy vs. resected samples for immunohistochemical staining is described in the Methods section as follows: We used endoscopic biopsy specimens in cases of initially metastatic patients, whereas resected samples were used in cases of recurrence after curative resection.

5. It is also puzzling that the tubulin expression did not correlate with any other parameters such as tumor grade, histology type etc. in this study.

   We agree. We have included your comments as a limitation of this study in the Discussion section as follows:

   → Third, TUBB3 expression did not correlate with other clinical parameters such as histological grade or Lauren classification.
Overall, this article contributed to the ongoing discussion about tubulin III as predictive or prognostic marker for a variety of tumor types. It adds value to the better understanding of the tumor biomarker and significance of tubulin family in relation to cancer treatment and response. The title and abstract accurately convey what has been found. I think the author should compare to some other similar studies in other tumors in the discussion. The paper is acceptable with minor modifications.

References to similar studies of other tumors have been added to the Discussion section as follows:

→ Several clinical studies have assessed the prognostic or predictive value of TUBB3 expression in patients with ovarian, cervical, or breast cancer. Most of these studies have shown that TUBB3 expression is associated with resistance to tubulin binding agents, a poor prognosis, or both [17]. Koh et al. [31] also reported that TUBB3-positive patients showed lower response rates, and that the PFS and OS times were shorter in patients with head and neck squamous cell carcinoma receiving induction chemotherapy.
Response to reviewer's report (reviewer: Diana English)

Major compulsory revisions
1. Another means of validating the TUBB3 and ERCC1 expression in samples would improve the quality of the paper such as use of RT-PCR instead of the use of only immunohistochemistry.

   Thank you for your comments. We attempted to perform RT-PCR after receiving the decision letter, but about half of the paraffin block samples were unavailable or not suitable for RT-PCR. Therefore, we were unable to follow up on your suggestion. We are very sorry for not performing RT-PCR.

2. Comments in the discussion section on other studies in the literature looking at the correlation of TUBB3 with clinical outcome in gastric cancer patients managed with other taxane based regimens e.g. (Taxanes and capecitabine) would add to the paper.

   In accordance with your suggestion, we have revised the Discussion section as follows:

   → Lu et al. [33] analyzed TUBB3 mRNA expression (as determined by real-time quantitative polymerase chain reaction) in patients with advanced gastric cancer receiving first-line paclitaxel plus capecitabine chemotherapy. They demonstrated that high-level TUBB3 expression was significantly associated with a lower response rate and shorter PFS and OS.

Minor essential revisions
1. The tumor samples examined for TUBB3 and ERCC1 expression were that of samples taken at diagnosis. I assume this means the primary diagnosis and as such one wonders if the recurrent tumors would be expected to have the same TUBB3 and ERCC1 expression?

   This concern and others are almost unavoidable in retrospective studies. Could the authors please comment?

   Thank you for your comment. It is possible that our immunohistochemical staining results for the pretreatment endoscopic biopsy specimens or resected samples did not correlate with those of the entire primary tumor or metastatic tissue.
We have included your comments as a limitation of this study in the Discussion section.

2. How did the authors ensure that no cases were missed in the medical record review?

Thank you for your comment. We selected patients whose paraffin wax-embedded tumor tissue and medical records were available. We enrolled patients only when both tumor tissue and medical records were available. We have added this information to Figure 1.

3. Following on the above point, can the authors include more of a discussion on the limitation of their study?

In accordance with your comment, we have revised the Discussion section as follows:

→ Despite demonstrating the predictive significance of TUBB3 expression, the present study has several potential limitations. First, it was a retrospective analysis from a single institution. Therefore, the chemotherapy dose and schedule might be different from patient to patient according to individual patient organ function, tolerability, and toxicity profiles. Second, this study included a somewhat heterogeneous patient population. Among 146 patients, 90 initially presented with metastatic disease, whereas 56 had recurrent disease after curative resection. Third, TUBB3 expression did not correlate with other clinical parameters such as histological grade or Lauren classification. Finally, it is possible that the immunohistochemical staining results of the pretreatment endoscopic biopsy specimens or resected samples did not correlate with those of the entire primary tumor or metastatic tissue.

Discretionary revisions
1. A comment on the need for a randomized control trial with determination of TUBB3 and ERCC1 status upfront followed by administration of treatment regimens may likely be one of the best approaches to identifying the true significance of these markers in prognosis for gastric cancer. Also a randomized trial may account for confounding variables not addressed in this study such as patient's performance status.
In accordance with your comments, we have revised the Discussion section as follows:

→ Additional prospective, randomized controlled trials are needed to identify the true significance of TUBB3 and ERCC1 in the prognosis of gastric cancer. Randomized clinical trials may also account for confounding variables such as patient performance status.