Peer Review File

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Reply to comments of reviewers

Reviewer A

Comment 1: Why to focus on the maximum tolerated dose of apatinib combined with irinotecan, not apatinib alone in the paper?

Reply 1: It was also a key issue when we designed the present study. Monotherapy of apatinib might be a choice for esophageal cancer patients who failed in standard treatment. But there were two questions concerning apatinib monotherapy as 2nd-line treatment. Firstly, there were only a few retrospective studies involved apatinib treatment in ESCC. And subjects enrolled were heterogeneous. The safety data of apatinib in ESCC was inadequate. Secondly, considering worsen performance status of patients who failed in 2nd-line treatment, only a few proportions of them had opportunity to receive further treatment. Thus, 2nd-line treatment is the key to improve survival of esophageal cancer. Chemotherapy is recommended 2nd-line treatment. Apatinib had been proved to treat gastric cancer based on data of previous clinical trials. And combining chemotherapy with anti-angiogenetic agents had been proved effective and safe in other cancers. Therefore, we designed combination treatment of apatinib and irinotecan in this study instead of monotherapy of apatinib. We hoped it might improve survival as well and benefit our subjects in ethic concern.

Changes in the text: The reason of combining apatinib and irinotecan treatment instead of apatinib monotherapy had been addressed as advised. (see line 94-100, page 6)

Comment 2: What is the role of VEGF and VEGFR in ESCC?

Reply 2: Previous studies had been revealed that VEGF over-expressed in 24-93% ESCC. And expression of VEGF/VEGFR was associated with microvessel density of ESCC tissue. VEGF was also reported of a prognostic factor of ESCC and a prospective predictive factor of chemoradiotherapy in ESCC. Thus, targeting VEGF or VEGFR was theoretically feasible to treat ESCC.

Changes in the text: Background of VEGF/VEGFR in ESCC had been added in the introduction section and 2 references (#3 and #4) had been added as advised. (see line 58-63, page 4)

Comment 3: Please supplement the introduction of 3+3 dose-escalation design, and research progress of apatinib and irinotecan in the introduction.

Reply 3: Thank you for your suggestion. We had increased background of dose-escalation design of phase 1 clinical trial and explain our concerns about introducing 3+3 design in present study.

Changes in the text: The background of 3+3 study design had been added in the introduction section as advised. (see line 110-120, page 6-7)

Comment 4: Please provide representative images of CT before and after treatment.

Reply 4: CT Images of patient #7 before and after treatment had been added as advised.

Changes in the text: A figure (Fig 2) had been added. (see line 260, page 13)

Comment 5: How many enrolled patients in the research? Please illustrate clearly in the methods or results.

Reply 5: Totally 12 patients had been enrolled and it had been addressed in the results section. (see line 204, page 11)

Changes in the text: None.

Comment 6: How to treat for occurred adverse events? How to determine the optimum dose of apatinib in the research?

Reply 6: Adverse events were treated according clinical routines. For example, leukopenia was treated by G-CSF and vomiting was treated by ondansetron and/or dexamethasone. The treatment of adverse events had been added in the Methods section. The optimum dosage of apatinib was identified by MTD. The MTD was defined as the highest dose at which no more than one of six patients experienced a DLT event in a certain arm. While the DLT was defined as any grade 4 hematological AEs or any grade 3–4 non-hematological AEs in the first three treatment cycles.

Changes in the text: The treatment of adverse event had been added in the methods section as advised. (See line 191-193, page 10)

The criteria of optimum dosage had been illuminated also in the methods section as advised. (See line 183-187, page 10)

Comment 7: Why to pay more attention to bleeding and thromboembolic events in using of VEGF or VEGFR inhibitors?

Reply 7: Activation of VEGFR2 promotes proliferation and migration of vascular endothelial cells. Blocking the VEGFR pathway will impair the function of endothelial

cells which would increase the risk of vascular-related adverse events. In previous studies, ramucirumab had been reported could increase any-grade hemorrhage in non-small cell lung cancer (Ref #26 and #27). Apatinib is a selective anti-VEGFR2 tyrosine kinase inhibitor. And there were case reports that apatinib resulted in bleeding (Ref #28 and #29) and thromboembolic events (Ref #18) in different cancers. It was the reason that we paid more attention to bleeding and thromboembolic events of apatinib in present study. We hoped to provide detail safety data of apatinib when treating ESCC. Background of VEGFR2 function, vascular-related adverse events of ramucirumab and apatinib in different cancers had been discussed in the discussion section. (see line 268-317, page 14-16)

Changes in the text: None

Comment 8: Whether the optimum dose of apatinib will be changed, when apatinib combined with other drugs?

Reply 8: Yes, we agree with the reviewer that the dosage of apatinib might be changed when combined with other cytotoxic agents. We illuminated it in the discussion section as advised.

Changes in the text: The possibility of changing apatinib dose when combined with other cytotoxic agents was added in discussion section as advised. (See line 340-341, page 17)

Comment 9: What are your suggestions for treatment for ESCC with apatinib? Please supplement in the discussion.

Reply 9: Thank you for your suggestion. Based on previous studies and our data of present study, we believed that as an anti-angiogenetic agent, apatinib had a potential anti-tumor activity to treat ESCC. We had increased discussion to explain it.

Changes in the text: The potential use of apatinib in ESCC had been discussed in the discussion section as advised. (See line 335-338, page 17)

Reviewer B

It is a valuable study with accurate methodology.

Few minor remarks to authors:

Comment 1: Line 121: > 3 months instead of 3 month

Reply 1: We are sorry for our carelessness. The error had been corrected. And we had also carefully checked spelling in whole manuscript to avoid typos.

Changes in the text: The spelling error had been corrected as advised. (See line 141, page 8)

Comment 2: line 137 to 148: it would be more accurate to separate non-inclusion criteria and exclusion criteria

Reply 2: It is a very important and helpful suggestion. We had rewritten the exclusion criteria as advised.

Changes in the text: Exclusion criteria had been rewritten as advised. (see line 157-170, page 9)

Comment 3: lines 176-177: there are data which are expressed as means and ranges

Reply 3: We had changed the expression of survival data as advised.

Changes in the text: Survival data were expressed as means and ranges as advised. (see line 265-266, page 14)

Comment 4: line 292: the (e was missing)

Reply 4: We are sorry for our carelessness. The error had been corrected. And we had also carefully checked spelling in the whole manuscript to avoid typos.

Changes in the text: The spelling error had been corrected as advised. (See line 311, page 16)