Author's response to reviews

Title: Establishment and identification of a rabbit model of peritoneal carcinomatosis from gastric cancer

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Author's response to reviews: see over
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Dear editor:

Thank you for your kind suggestions regarding the revision our manuscript “Establishment and identification of a rabbit model of peritoneal carcinomatosis from gastric cancer” (MS: 2225066522825128).

As two reviewers, Dr. Sachio Fushida and Dr. Andrea McCart, have no further comments, our second revision has focused on the comments by Dr. Jens Hartmann.

The following is the point-to-point response to comments by Dr. Jens Hartmann:

1. Language was checked and considerably improved. However there are still many grammar and typing mistakes. Again, a revision by a native speaker is recommended.

Response: We have done our best to check and correct grammar and typing mistakes and have invited a native English speaker to finalize our manuscript. Still, we would appreciate it very much if language editors of BMC CANCER could add further finishing touches to our manuscript.

2. The authors mention, that histopathological studies of tumour tissues from rabbits of all groups and time points showed metastatic disease. It still remains unclear however, how can the authors use squamous tumour cells create a
tumour model of peritoneal carcinosis of gastric cancer, which is typically an adenocarcinoma, particularly a tumour from different biological and histopathological behaviour. This remains a weak component of the study.

Response: We agree with the reviewer, to some extent, that this VX2 tumour model may not exactly the same as clinical patients, because it is squamous cell carcinoma. This may be a weak component of the study. However, we are also aware of the fact that VX2 tumour has been used as liver cancer model (typically adenocarcinoma) (Ref 42 and 43), colon cancer model (typically adenocarcinoma) (Ref 44 and 45) and bone tumor model (typically sarcoma) (Ref 50), to name just a few examples. These cancers are not squamous cell carcinoma, but VX2 tumour can still show typical features of these tumours.

3. The endpoint and main parameter of the study is not clearly to identify. Establishment of a PC rabbit model as suggested in the conclusion paragraph? Simulation of advanced gastric cancer? Study of the progression and metastatic behaviour? Best technique to induce PC?

Response: The endpoint of the study is clearly stated in the Background section of the Abstract. This study was to establish a stable rabbit peritoneal carcinomatosis model of gastric cancer……and to analyze the clinico-pathological features.

4. The manuscript should be condensed concentrating on the main objective of
Response: The manuscript has been condensed and main objectives have been clarified. In particular, we have taken Dr. Hartmann’s suggestions (comments 7 and 19) that the whole topic of CT scans have been omitted. This makes the manuscript much condensed.

5. The absence of a control group and the small sample size weakens the study.

Response: Our objective of this study is to establish a large animal model of peritoneal carcinomatosis. This is not an interventional study. It is not to test the efficacy of a treatment. A control group is not needed.

6. Paragraph ‘Methods - animal observation …’: Which were the exact parameters for euthanizing animals in group B and C? A direct comparison of tumour development between the groups is not possible. Why did the investigators chose this setting? The exact postoperative ‘survival’ time must be provided because in group B+C might be euthanized much earlier than in group A – this fact has an tremendous impact on the evaluation of the different techniques and success in PC induction and the lack of lung metastases.

Response: In this study, we did not wait for the natural death of the animals. As a result, the exact postoperative ‘survival’ time was not available in our study. According to the regulations of the Animal Experimental Center of Wuhan University, and UKCCCR Guidelines for the welfare of animals in experimental
neoplasia [Cancer and Metastasis Reviews. 1989;8(1):82-88], we must implement humane end points rather than the natural death. Therefore, animal euthanasia was performed according to the study protocol and before these animals became moribund.

7. Paragraph ‘Methods - computed tomography to monitor …’: It is not easily to understand: week by week in 3 animals a CT scan was performed. Simultaneously 3 animals of this group were euthanized. Does it mean that the CT scans were performed always in the same 3 animals? Describing the CT scanner probably the Siemens sensation is meant. A 12F-tube must have an ID of 4mm. The information within the brackets is redundant an should be omitted.

Response: This part has been omitted in the revised text, as suggested by Dr. Hartmann (comment 19: whole topic of CT scans should be omitted).

8. Statistical analysis: The use of non-parametric tests is required. Small and over the time decreasing sample sizes ague against normal distributions. Confidence intervals are missing. Corrections for multiple testing are needed when comparing intragroup differences over the time (i.e bodyweight, tumoursize. When comparing very small groups (n=3) p-values are misleading and should be avoided or interpreted very cautiously. The used statistical analysis is inappropriate. It should be corrected.
Response: All statistical analyses have been carefully revised and corrected. Fisher exact test was performed.

9. Paragraph ‘Results – success of PC model …’: 3 early deaths in group C and 3 animals without developing PC – this are 6 of 12. You refer to 7/12 – please explain.

Why was in at least 4 animals a fluid (lethal) rehydration necessary? That is not common after ip-injections or minor surgery.

Response: There is a typing mistake in the description of animal death. “2 rabbits each in Groups B and C died of ……” should be “1 rabbit each in Groups B and C died of…… The calculation itself is correct.

10. Paragraph ‘Results – tumor growth …’: An acceleration of tumour growth cannot be deduced from this trial. You compare different animals on different time points (again: n=3?).

Response: This is not a clinical trial. Rather, it is an experimental study on highly homogenous animals performed in standard laboratory conditions. After animal euthanasia at set time points, the tumor size was carefully measured. From such calculation, we know that the tumour shows accelerated growth.

11. Table 1: Again: are p-values corrected for multiple testing and use
non-parametric tests. Please provide in the legend, that these data are from group A animals and the number of scanned animals (3?).

Response: We have taken Dr. Hartmann’s suggestions and omitted this table. Instead, we described in the text the increases in tumor size at different time points.

12. An acceleration of loosing body mass is not visible after the second week. Again: please use non-parametric tests.

Please provide the data of group B+C animals. Did they suffer from an equally fast deterioration?

Response: Animals in the three groups show similar weight changes. As the diagram of weight changes have been removed, we described the weight changes in the text.

13. Figure 2: The y-scale should start at 0. The number of animals must be provided for each point of time (at W4 only 3 rabbits!). Are p-values corrected for multiple testing?

This diagram of a very small and rapidly decreasing number of animals seems to be inappropriate and dispensable for the objective of the study. It should be omitted.

Response: We have taken Dr. Harmann’s advice and deleted this diagram from the second revision.
14. Paragraph ‘Results – growth characteristics …’: It is generally problematic to deduce a development from the investigation in 3 animals per time point. It is stated that tumours in group B+C showed the same growth character. Because all animals in these groups were euthanized at the time of ‘detoriation’ a development of tumour growth could not be investigated. This should be stated clearly.

Response: We have taken Dr. Hartmann’s advice and made this point clear in the second revision.

15. Figure 3: Please provide the group.

Response: We have done so in the second revision.

16. Figure 4: Please provide the group.

Response: We have done so in the second revision.

17. Paragraph ‘Results – histopathological …’: What does ‘sacrifice after euthanasia’ mean? 2 rabbits after 2 weeks? It would be better generalized: all investigated tumour specimen showed …

The last sentence suggests that specimen from animals were studied at different points of time. This suggestion should be avoided.
Response: We have taken Dr. Hartmann’s advice and revised this part as suggested.

Below is the revised text:

All investigated tumor specimens showed extensive invasive growth and tissue destruction. The tumors, on the greater curvature of the gastric antrum, penetrated the mucosal layer to form ulcers. Microscopic view could find cancer nests penetrating the whole stomach wall, with typical invasion into the muscle layer and the gastric glands (Figure 4A). The tumor cells are round, oval or atypical morphology with many pathological mitotic figures (Figure 4B). There were also conspicuous lymphocytes, plasma cells and other inflammatory cells infiltration.

18. Table 2: The table shows results for CT scans in group B and C. In contradiction in the methods paragraph ‘computed tomography to monitor…’ it is mentioned, that only group A animals underwent CT investigations.

Response: As suggested by Dr. Hartmann, we have omitted the CT scan section from table 2 in the second revision. And as we have deleted the previous Figure 1 in the second revision, the previous figure 2 becomes figure 1 in the new version.

Below is the revised Table 1.

| TABLE 1. Tumor characteristics of three different inoculation approaches |
|--------------------------------------------------|----------------|----------------|----------------|
|                  | Group A | Group B | Group C          |
| Technical         | Laparotomic | Laparotomic | Percutaneous |

<table>
<thead>
<tr>
<th>feature</th>
<th>orthotopic tumor cell injection into the gastric submucosa</th>
<th>orthotopic tumor tissue inoculation beneath the gastric antrum</th>
<th>tumor cell injection into the peritoneum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Success rate</td>
<td>100% (12/12)</td>
<td>91.7% (11/12)</td>
<td>58.3% (7/12)</td>
</tr>
<tr>
<td>Major pathological events</td>
<td>Rapid tumor progression resulting in respiratory distress syndrome, diffused peritonitis due to perforation of gastric tumor, intestinal obstruction, renal failure</td>
<td>Rapid tumor progression resulting in intestinal obstruction, renal failure</td>
<td>Rapid tumor progression resulting in intestinal adhesion, an obstruction, renal failure</td>
</tr>
<tr>
<td>Gross pathology</td>
<td>Ulcerative gastric cancer with PC, ascites</td>
<td>Ulcerative gastric cancer with PC, ascites</td>
<td>PC without gastric ulcer, ascites</td>
</tr>
<tr>
<td>Histopathology</td>
<td>Penetrating growth of cancer cell nests invading surrounding structures, tumor necrosis in the central zone of the tumor mass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Advantages</td>
<td>Most resemble clinical gastric cancer with PC</td>
<td>Technically less difficult</td>
<td>Technically easy</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Technically difficult</td>
<td>Not exactly mimic gastric cancer with PC</td>
<td>Mistaken injection into the intestines</td>
</tr>
</tbody>
</table>

19. Paragraph ‘Results – medical imaging …’: This paragraph does not give additional information, is not mentioned in the discussion but there are several problems in understanding (see point 6+17 of this review). Focusing on the (certainly not exactly given) main objective of the study this paragraph and whole topic of CT scans should be omitted.
Response: We have taken Dr. Hartmann’s advice and omitted the whole topic of CT scans.

20. Paragraph ‘Discussion’: As already mentioned: the evaluation of the course of development of the tumour is problematic because of the very small sample size of 3 animals per point of time.
A comparison of the different groups is also nearly impossible because of the different (and for the reviewer unknown) postoperative survival times.

Response: As stated earlier, this is not a clinical trial. Rather, it is an experimental study on highly homogenous animals performed in standard laboratory conditions. After animal euthanasia at set time points, the tumor size was carefully measured. Such calculations could show the tumor growth pattern. From the standpoint of animal welfare, it is not advisable to have a definite postoperative survival time in this study. Therefore, we did not include this parameter in this study.

We hope these point-to-point responses to Dr. Hartmann’s kind suggestions could make the manuscript clearer, more condensed and up to your standard.

Thank you again for your kind assistance, and also special thanks to Dr. Hartmann, Dr. Fushida and Dr. McCart for their time input and valuable suggestions.
With kind regards,

Yours Sincerely,

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China