Peer Review File

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Reviewer A

There were a few reports discussing about ramucirumab plus paclitaxel after nivolumab plus chemotherapy. Clinical data in this study are meaningful.
However, I found several points you should reconsider.

1. Clinical outcomes in your study patients were poor compared to RAINBOW study. The sample size is small. There is a possibility of selection bias. It is difficult to conclude ramucirumab plus paclitaxel is insufficient.

Answer 1:
Thank you for your comment.
We agree on your opinion. According to your commnets, we changed the sentence in the part of concolusin of Abstract and Discussion as follows; (see Page 3, line 64, page 11, line 235).

This analysis suggested that ramucirumab plus paclitaxel as second-line therapy might be further studied in advanced gastric cancer patients after failure of nivolumab plus chemotherapy.

2. Dr Sasaki reported that ICI enhanced the efficacy of ramucirumab plus paclitaxel (ESMO Open. 2020 Jul;4(Suppl 2):e000775. doi: 10.1136/esmoopen-2020-000775.). You should discuss about this difference.

Answer 2: Thank you for your comment.
According to your comments, we added the sentence in the part of the discussion as follows; (see Page 10, line 220).

Sasaki et al. reported that anti-PD-1 therapy exposure in advanced gastric cancer enhanced the efficacy of ramucirumab plus paclitaxel. ORR of ramucirumab plus paclitaxel was 60.6% in the anti-PD-1 exposed group and 20.0% in the anti-PD-1 naïve group ($p<0.001$) (22). The study showed an favorable RR compared to the present study and the RAINBOW study, however, the median PFS in this study was also 3.7 months in the anti-PD-1 exposed group and 3.3 months in the anti-PD-1 naive group. Studies for ramucirumab plus paclitaxel after the immunotherapy have reported inconsistent findings. Further research for this area is needed.

3. I suppose that red line in Kaplan-meier curve of Figure 3 might be responder group.

Answer 3: Thank you for your pertinent point.
According to your comment, we changed it as follows; The legends in figure 2 and figure 4

have been modified because the notations for responder and non-responder are reversed.
Figure 2

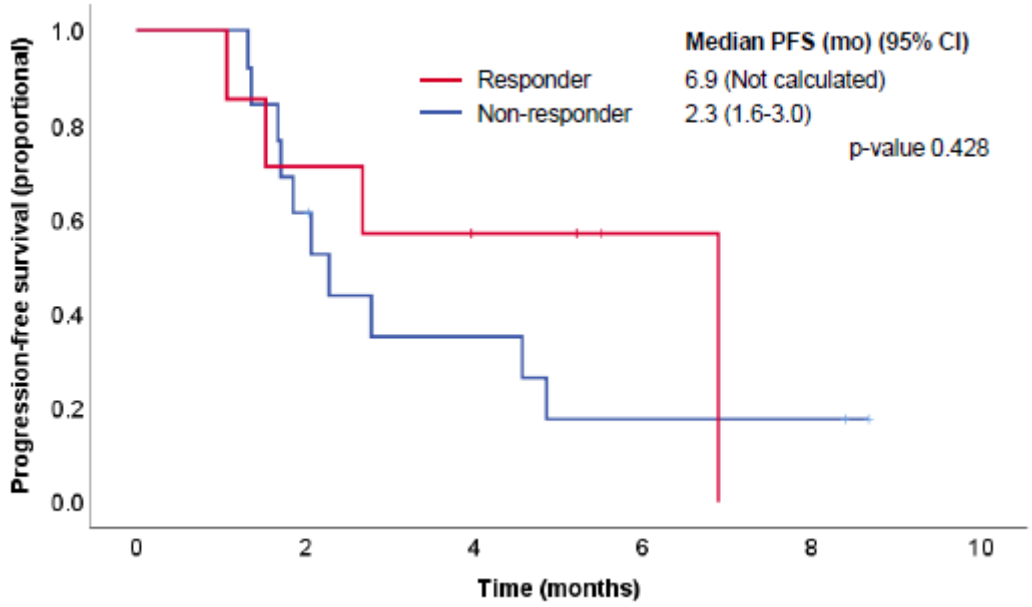
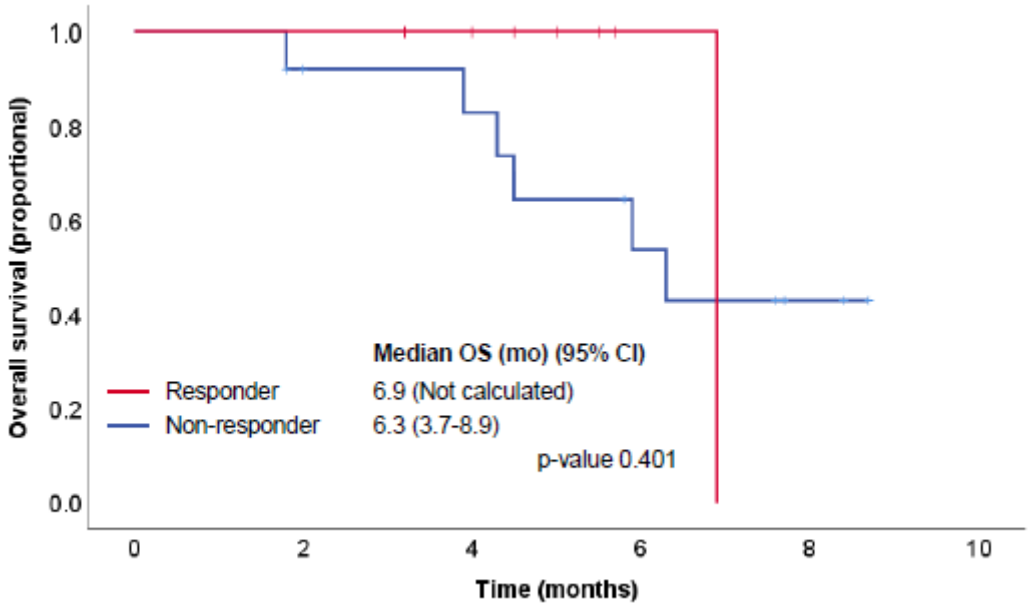


Figure 4



Reviewer B

The role of ramucirumab plus paclitaxel as second-line therapy afterfailure4 of nivolumab plus doublet chemotherapy

Abstract:

1. Why was (95% confidence interval, not calculated)?

Answer 1:Thank you for your comment.
According to your comment, we rewrite it as follows; (see Page 3, line 60).

6.9 months (95% confidence interval, xx- not reached)

2. Please clarify how you define responders and non-responders?

Answer 2: Thank you for your comment.
We added the definitions of responder and non-responder in the part of method as follows; (see Page 7, line 123, line 133).

The ORR is defined as the percentage of patients who achieve a response, which can either be complete response (CR, complete disappearance of lesions) or partial response (PR, reduction in the sum of maximal tumor diameters by at least 30% or more). The DCR is defined as the percentage of patients who have achieved CR, PR and stable disease (SD).

Responders were defined as patients whose CR or PR was confirmed in response evaluation performed during first-line nivolumab plus chemotherapy treatment, and non-responders were defined as patients whose best ORR was SD or PD.

3. What is partial response in this case?

Answer 3:
Thank you for your comment.
All patients were evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Partial response was defined when reduction in the sum of maximal tumor diameters by at least 30% or more

Methods:

1. What is p on line 94?

Answer1: Thank you for your kind comment. The typo has been corrected.

2. Rationale for the sample size, Was any power analysis done?

Answer 2: Thank you for your comment.
This analysis was a retrospective study. We described the limitation of this point as follows; (see Page 11, line 228).

There are a few limitations to this study. It was a retrospective study with a clinically

diverse group which may have been biased. Second, the study included only a few patients, making it challenging to reach definitive conclusions on genomic biomarkers. Third, the study only included Asian patients with mAGC, limiting the generalizability due to the different molecular profiles and clinical characteristics between Western and Eastern patients with AGC. Therefore, our findings must be interpreted with caution. Nevertheless, this analysis suggested that ramucirumab plus paclitaxel as second-line therapy might not be sufficient in AGC patients after failure of nivolumab plus chemotherapy.

3. Why were baseline characteristics not controlled for in the analysis for correlations?

Answer 3: Thank you for your comment.
This analysis was a retrospective study. And the study included a relatively small sample size. Thus, no additional controls were performed in the correlation analysis. Currently, we conducted the prospective clinical trial in patients with the failure to nivolumab plus chemotherapy.

Results:

1. Define what is a partial response.

Answer 1:
Thank you for your comment.
Partial response (PR) is defined as at least a 30% decrease in the sum of the target lesions according to the RECIST 1.1 criteria. (see Page 7, line 123).

The ORR is defined as the percentage of patients who achieve a response, which can either be complete response (CR, complete disappearance of lesions) or partial response (PR, reduction in the sum of maximal tumor diameters by at least 30% or more). The DCR is defined as the percentage of patients who have achieved CR, PR and stable disease (SD).

2. Criteria used for disease control rate?

Answer 3: Thank you for your comment.
For this point, we described the sentence in the part of Method as follows; (see Page 7, line 123).

The ORR is defined as the percentage of patients who achieve a response, which can either be complete response (CR, complete disappearance of lesions) or partial response (PR, reduction in the sum of maximal tumor diameters by at least 30% or more). The DCR is defined as the percentage of patients who have achieved CR, PR and stable disease (SD).

3. Mention about TREND checklist in the methods and how it was used in drafting this study.

Answe 3: Thank you for your comment.
We added the sentence as follows; (see Page 7, line 139).

The basis for reporting this study was the TREND statement checklist for Transparent Reporting of Evaluations with Nonrandomized Designs.

Reviewer C

The article entitled "The role of ramucirumab plus paclitaxel as second-line therapy after failure of nivolumab plus doublet chemotherapy" presents a retrospective study on the efficacy of paclitaxel-ramucirumab in patients with gastric cancer after first-line chemo-immunotherapy. The manuscript deals with a topic that is currently vacant in oncology, namely the second-line treatment of gastric cancer after the recent introduction of immunotherapy in the first-line setting. This is undoubtedly a topic of great interest and despite the limited sample size may be interesting for publication in the journal. There are currently no articles on real-life data or clinical trials that clarify the performance in this field.

In making an overall revision of the article, the authors will undoubtedly need to make a number of major and minor changes in order to consider publication of the manuscript. The title, the abstract and the different sections of the article need several major changes. Furthermore, the methodology that the authors have used is the correct one to answer the questions that are being asked and is therefore a point in favor of the article. The language is also correct, and I believe it needs no corrections. The figures and tables are also what the article needs and do not require changes.

The following are the changes that I believe are necessary for the article:
Major changes

- Methods: This section should be done better. It should be differentiated into different subsections such as inclusion and exclusion criteria, statistical analysis, variables collected for the study, objectives, etc. Furthermore, the terms OS, PFS, etc. should be correctly defined. Also indicate how toxicity was collected, which he imagined would be according to the CTCAE criteria.

Answer: Thank you for your comment.
We rewrote the part of method as follows; (see Page 6, line 119).

Clinical outcomes were evaluated for objective response rate (ORR), disease Control Rate (DCR), progression-free survival (PFS), overall survival (OS), and safety. The ORR and DCR was assessed by investigators, according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 based on computed tomography (CT)

or magnetic resonance imaging (MRI). The ORR is defined as the percentage of patients who achieve a response, which can either be complete response (CR, complete disappearance of lesions) or partial response (PR, reduction in the sum of maximal tumor diameters by at least 30% or more). The DCR is defined as the percentage of patients who have achieved CR, PR and stable disease (SD). Treatment-related adverse events (TRAEs) were assessed and graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 5.0. PFS was defined as the time from the start of ramucirumab plus paclitaxel until the date of disease progression or death from any cause. Patients who remained alive at the analysis cutoff date were censored at that time. OS was defined as the time from the start of ramucirumab plus paclitaxel until death from any cause, with patients alive at the time of the last data cutoff and at the time of the last follow-up being censored. Responders were defined as patients whose CR or PR was confirmed in response evaluation performed during first-line nivolumab plus chemotherapy treatment, and non-responders were defined as patients whose best ORR was SD or PD.

- Results: What were the PD-L1 values per CPS for the treatment? It would be interesting to analyse the results also in the second-line according to these values and to specify which ones were used for the first line.

Answer:
Thank you for your comment.
We added the data for PD-L1 value in Table 1.
Revised Table 1 is as follows;

Table 1 Baseline characteristics

	N=20	%
Age		
Median (range)	56	41-76
Sex		
Male	13	65.0
Female	7	35.0
ECOG performance at 2L start		
1	20	100.0
Primary tumor site		
Gastric cancer	20	100.0
Disease status		
Advanced	15	75.0
Recurrent	5	25.0
Histological type		
Tubular adenocarcinoma		

Moderate	3	15.0
Poorly	8	40.0
Poorly cohesive carcinoma	9	45.0
Microsatellite instability status		
Microsatellite stable	20	100.0
PD-L1 CPS		
<1	7	46.7
≥1	8	53.3
N.E.	5	
Number of organs with metastasis		
1	4	20.0
2	9	45.0
3	4	20.0
4	3	15.0
Sites of metastases		
Lymph nodes	15	75.0
Peritoneum	13	65.0
Lung	3	15.0
Liver	3	15.0
Ovary	3	15.0
Previous chemotherapy regimen		
Nivolumab + XELOX	15	75.0
Nivolumab + FOLFOX	5	25.0

N.E: Not evaluated, XELOX: capecitabine and oxaliplatin, FOLFOX: fluorouracil, leucovorin, and oxaliplatin

Additionally, we tried to analyze survivals including PFS and OS according to CPS, but we can't get the meaning data because of too small sample size.

- Discussion: give a more theoretical explanation as to why this combination is not effective after chemo-immunotherapy and why it is effective if immunotherapy is not used in the first-line.

Answer: Thank you for your comment.
I think that this comment is very important to improve the treatment outcomes in metastatic gastric cancer in the immuno-oncologic era.

For this point, we described some in the part of Discussion (see Page 10, line 212).

The immune checkpoint inhibitor and the anti-angiogenic agents are important stromal agents. These stromal agents made changes in tumor microenvironment(TME). Theologically, the effect of nivolumab plus cytotoxic chemotherapy infkuenced on the TME including immune cells and tumor vasculature. Also, the refractoryness to nivolumab and cytotoxic chemotherapy might make changes the TME. This change at the time of refractoryness to nivolumab plus chemotherapy might affect the antitumor effect of ramucirumab and paclitaxel. Further studies for the TME after the immune checkpoint inhibitor and/or the anti-angiogenic agents are needed.

- Discussion: since the study population is Asian, the results could also be compared with the RAINBOW-Asia clinical trial. This study is easier to compare with the manuscript than the RAINBOW clinical trial.

Answer: Thank you for your comment.
According to your comment, we added the RAINBOW-Asia results in the discussion as follow; (see Page 9, line 188).

The RAINBOW trial reported that the median PFS was 4.4 months (95% CI, 4.2- 5.3) and the median OS was 9.6 months (95% CI, 8.5- 10.8) for all enrolled patients. The present study showed relatively shorter survival outcomes compared to those in the RAINBOW trial. In the RAINBOW-Asia trial, the median PFS was 4.14 months (95% CI, 3.7-4.3) and the median OS was 8.71 months (95% CI, 7.98-9.49) in the ramucirumab plus paclitaxel group. (10) However, patients achieving tumor response to nivolumab plus chemotherapy as the first-line had comparable survival outcomes to ramucirumab plus paclitaxel compared to those in the RAINBOW and RAINBOW-Asia trials (5,10). These findings suggest the possibility of selectively applying ramucirumab and paclitaxel to patients with AGC as second-line therapy, depending on tumor response before combining nivolumab and chemotherapy. However, with the small

number of single-center studies, additional prospective studies are considered necessary.

Minor changes
- Title: specify that this is a study in gastric cancer.

Answer. Thank you for the comment.
We corrected it as follows; (see Page 1, line 2).

The role of ramucirumab plus paclitaxel as second-line therapy after failure of nivolumab plus doublet chemotherapy in patients with advanced gastric cancer

- Abstract: the recruitment time should be moved from results to methods.

Answer. Thank you for the comment.
We corrected it as follows; (see Page 3, line 48).

Twenty patients who progressed on nivolumab plus chemotherapy as first-line therapy were treated with ramucirumab plus paclitaxel between December 2021 and September 2022.

- Abstract: add sex in the results.

Answer. Thank you for the comment.
We added it as follows; (see Page 3, line 51).

and 13 (65.0%) were male.

- Keywords: add "second-line".

Answer. Thank you for the comment.
We added it as follows; (see Page 4, line 68).

Keywords: Advanced gastric cancer, Chemotherapy, Nivolumab, Paclitaxel, Ramucirumab, Second-line

- Introduction, line 67: give specific data on mortality.

Answer. Thank you for the comment.
We added it as follows; (see Page 5, line 72).

According to GLOBOCAN 2020 estimates, stomach cancer was the fifth most common malignancy in the world, with approximately 1.1 million new cases in 2020, and the fourth leading cause of cancer death with approximately 800,000 deaths (accounting

for 7.7% of all cancer deaths) (1).

- Introduction: human genes should be in italics.

Answer. Thank you for the comment.
We corrected them.

- Introduction: give the name of the clinical trial of first-line chemo-immunotherapy and add some efficacy data on this trial.

Answer. Thank you for the comment.
We added it as follows; (see Page 5, line 87).

Recently, nivolumab has shown superior survival benefits and an acceptable safety profile when combined with fluoropyrimidines and platinum as first-line therapy in previously untreated patients with AGC. In the CheckMate 649 study, combining nivolumab with chemotherapy significantly improved overall survival (hazard ratio (HR) 0.71, 98.4% CI 0.59-0.86, p<0.001) and progression-free survival (PFS, HR 0.68, 98% CI 0.56-0.81, p<0.001)(6) compared to the chemotherapy group in treatment-naive AGC patients with a PD-L1 CPS of five or more (6).

- Methods, line 94: correct the P above the text.

Answer. Thank you for the comment.
We removed P

- Results: in Table 1 there is a data on ECOG different from the text. Correct it.

Answer. Thank you for the comment.
We corrected it as follows;

All patients had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 1 at the date of second-line chemotherapy.

- Results: No side effects related to the anti-angiogenic effect of ramucirumab such as hypertension, bleeding or thrombosis? Specify.

Thank you for your cimment. We added the sentence as follows; (see Page 9, line 174).

No side effects related to the anti-angiogenic effect of ramucirumab, such as hypertension, bleeding, or thrombosis, were identified.

- Conclusion: this point should be more open and less categorical.

Thank you for your comment.
We agree on your opinion. According to your commnets, we changed the sentence in the part of concolusin of Abstract and Discussion as follows; (see Page 11, line 235).

This analysis suggested that ramucirumab plus paclitaxel as second-line therapy might be further studied in advanced gastric cancer patients after failure of nivolumab plus chemotherapy.

In summary, I believe that the authors have done a good effort that is publishable if the changes specified above are made correctly. Undoubtedly, this study can be of great value to the scientific community to better understand second-line gastric cancer.