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Peer Review File

Dalpicilib combined with cetuximab in patients with HPV-negative, anti-PD-1-resistant recurrent or metastatic head and neck squamous cell carcinoma: A phase II trial

Corresponding Author: Dr Jingzhou Hu

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The study addresses a significant clinical need and presents encouraging efficacy and safety data for dalpiciclib plus cetuximab in a challenging patient population. While the single-arm design and limited biomarker sample size restrict definitive conclusions, the results are hypothesis-generating and relevant for future research. I recommend revision, with a focus on:
1. Deeper correlative biomarker analyses (linking genomic features to outcomes):
• I recommend the authors perform detailed subgroup analyses to determine if specific genomic alterations—such as TP53 mutation, CCND1 amplification, or their combination—are associated with differential response rates, PFS, or OS. Given the established roles of these alterations in HNSCC biology and therapy resistance, such analyses would provide valuable insight into potential biomarkers of response to dalpiciclib plus cetuximab.
• Additionally, please explore whether patients with CCND1 amplification or other cell cycle pathway alterations had higher response rates to this regimen, as this could support a precision medicine approach for future studies.
2. More detailed reporting and interpretation of immune cell changes:
• The authors should, if possible, provide more granular data on immune cell subsets—such as CD8+ T cells, Tregs, and natural killer (NK) cells—rather than reporting only total lymphocyte percentages. These subsets play distinct and sometimes opposing roles in anti-tumor immunity and are well-established as prognostic and predictive biomarkers in HNSCC.
• Please clarify whether changes in immune infiltration (such as the CD8+/Treg ratio or NK cell abundance) were associated with clinical outcomes in your cohort.
3. Clearer discussion of the limitations and the exploratory nature of biomarker findings:
• The authors should highlight the exploratory nature and potential significance of findings such as KCNMA1, TNXB, and TPCN2 alterations in nonresponders. While these genes may plausibly contribute to resistance mechanisms through known signaling pathways, their roles as biomarkers in HNSCC or in the context of CDK4/6 inhibitor therapy are not established in the current literature.
• Please also discuss limitations such as the small biomarker cohort, lack of functional validation, and the need for independent confirmation.
4. Improved contextualization with existing literature and justification of study design choices:
• The manuscript would benefit from more direct comparison to outcomes with other regimens (e.g., cetuximab monotherapy, other CDK4/6 inhibitors), as well as a rationale for the single-arm design and chosen dosing strategy.
If these revisions are addressed, the manuscript will make a valuable contribution to the field and provide a strong foundation for future research in this area.

Reviewer #2

(Remarks to the Author)
This paper explore Dalpicilib in combination with cetuximab after failure of anti-PD1.
This is a small monoarm study well conducted.
We observe a clear signal of efficacy with high response rate and promising PFS and OS even if it is difficult to be confident

in a mOS of 17 months with only 9.2 months of median PFS.
This drug remains toxic with 85% of patinets who need to reduce the dose: in further studies perhaps the schedule/dosage should be modified.

In the discussion the response rate to cetuximab alo,e after anti-PD1 is 20% in the interlink study and this level should probably be indicated. But clearly the combination alpicilib/cetuximab seems better.

Reviewer #3

(Remarks to the Author)
1. Although the protocol stated that the full analysis set (FAS) is to consist of "all enrolled subjects who have taken the study drug at least once", the study's analysis population apparently excludes 2 withdrawals (Figure 1B). Unless they withdrew consent, they should be included in the analyses. They can be used for OS analyses regardless.

2. The statement in the Introduction, "The incidence of human papillomaviruses (HPV)-negative HNSCC is higher than that of HPV-related HNSCC, which is often associated with lower response rate and poorer survival with immunotherapy.", seems reversed.

3. The 95% confidence interval for PFS extends down to 0. This seems unlikely and should be checked.

4. The legend of Fig. 4A) should include descriptions of the two marginal bar charts.

Congratulations on these important results.

R. Chappell, FASA, FSCT
Professor, Dept. of Biostatistics and Medical Informatics
University of Wisconsin School of Medicine and Public Health
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Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have addressed the previous concerns thoughtfully, and the noteworthy results are clearly presented. The work holds significance for the field and is well positioned in relation to the established literature. The conclusions are well supported by the data, and the methodology meets the expected standards, with sufficient detail to allow reproducibility.

There are no major flaws in the data analysis or interpretation that would prevent publication. Any questions/ suggestions previously raised have been adequately addressed.

In summary, I find the manuscript suitable for publication.

Reviewer #2

(Remarks to the Author)
OK now

Response to Reviewers

Reviewer(s)' Comments to Author:

Reviewer #1 (Remarks to the Author):

The study addresses a significant clinical need and presents encouraging efficacy and safety data for dalpiciclib plus cetuximab in a challenging patient population. While the single-arm design and limited biomarker sample size restrict definitive conclusions, the results are hypothesis-generating and relevant for future research. I recommend revision, with a focus on:

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• Additionally, please explore whether patients with CCND1 amplification or other cell cycle pathway alterations had higher response rates to this regimen, as this could support a precision medicine approach for future studies.

Re: Thank you very much for your valuable suggestion. We added the biomarker analyses on TP53 mutation and abnormal CDK4 related genes (CDNK2A deletion and CCND1 amplifications). For TP53 mutation analysis, no significant differences in ORR (66.7% vs 58.8% (Z=-0.26, P=0.798)), median PFS (14.3 vs 7.0 months (HR 0.28 [95% CI 0.08−1.11], P=0.127)), and OS (unreached vs 17.67 months (HR unevaluated, P=0.208)) were observed (Supplemental data 2E, F).

Interestingly, we found contrary results in abnormal CDK4 related genes analysis compared to prior studies. In our study, patients without abnormal CDK4 related genes had higher response and better prognosis compared with those with

abnormal CDK4 related genes (ORR was 81.8% vs 33.3% (P=0.028), median PFS was 14.3 vs 4.3 months (HR 0.26 [95% CI 0.07−0.91], P=0.035), and median OS was unreached vs 8.5 months (HR 0.23 [95% CI 0.05−1.06], P=0.060)). Similar phenomena were also observed in patients with only CCND1 amplification (Supplemental data 2A-D).

We re-checked our NGS sequence and confirmed the grouping information was correct. We analysed that CDK4/6 has been activated since p16(INK4a) was upstream of CDK4/6 and inactivated in HPV negative patients, CDNK2A deletion or CCND1 amplifications might facilitate the progression of the cancer cell cycle from G1 to S phase through bypassing CDK4/6 pathway. In the previous study, Clark AS et al revealed that palbociclib, a selective CDK4/6 inhibitor, was not effective at treating nonbreast solid tumors with either CCND1, 2, or 3 amplification.[1] Oppelt PJ et al also showed that HPV-negative, locally advanced HNSCC patients with CCND1 amplification had a lower response (16.7% vs 50%) towards palbociclib compared with those without CCND1 amplification.[3] However, these results need further investigation and confirmation in the future study.

References

1. Clark AS, Hong F, Finn RS, DeMichele AM, Mitchell EP, Zwiebel J, Arnaldez FI, Gray RJ, Wang V, McShane LM, Rubinstein LV, Patton D, Williams PM, Hamilton SR, Copur MS, Kasbari SS, Thind R, Conley BA, Arteaga CL, O'Dwyer PJ, Harris LN, Chen AP, Flaherty KT. Phase II Study of Palbociclib (PD-0332991) in CCND1, 2, or 3 Amplification: Results from the NCI-MATCH ECOG-ACRIN Trial (EAY131) Subprotocol Z1B. Clin Cancer Res. 2023 Apr 14;29(8):1477-1483.
2. Oppelt PJ, Ley JC, Puram SV, Jackson RS, Paniello RC, Rich JT, Pipkorn P, Liu J, Thorstad WL, Adkins DR. Palbociclib, a Selective CDK4/6 Inhibitor, Administered Before and After Chemoradiotherapy for Human Papillomavirus-Negative, Locally Advanced Head and Neck Squamous Cell Carcinoma: A Single-Arm, Phase II Trial. JCO Precis Oncol. 2025 May;9:e2500069.

2. More detailed reporting and interpretation of immune cell changes:

• The authors should, if possibxle, provide more granular data on immune cell subsets—such as CD8+ T cells, Tregs, and natural killer (NK) cells—rather than reporting only total lymphocyte percentages. These subsets play distinct and sometimes opposing roles in anti-tumor immunity and are well-established as prognostic and predictive biomarkers in HNSCC.

• Please clarify whether changes in immune infiltration (such as the CD8+/Treg ratio or NK cell abundance) were associated with clinical outcomes in your cohort.

Re: Thank you so much for your valuable advice. During this trial, we made an incidental observation: among patients who received dalpiciclib plus cetuximab, the two who responded to this regimen subsequently achieved a partial response (PR) to anti-PD-1 rechallenge therapy, whereas the two non-responders to dalpiciclib plus cetuximab showed no response to subsequent anti-PD-1 treatment. We then analyzed lymphocyte percentages in blood samples from enrolled patients and found preliminary evidence that responders exhibited a significant increase in lymphocyte percentages at the time of best response compared with baseline, while no significant changes in lymphocyte percentages were observed in non-responders.

Regrettably, due to insufficient attention to sample preservation during the trial, we did not retain post-treatment blood or tissue samples. This has made it extremely challenging to compare pre- and post-treatment changes in the immune microenvironment—a limitation we acknowledge.

To address your request for more granular immune cell analysis, we have supplemented the revised manuscript with an analysis of the relationship between baseline immune cell subsets ($CD8^+$ T cells, regulatory T cells [Tregs], natural killer [NK] cells) and clinical outcomes. Specifically, we examined associations between baseline $CD8^+$ T cell counts, Treg counts, NK cell counts, and the $CD8^+$/Treg ratio with treatment response, progression-free survival (PFS), and overall survival (OS) in patients receiving dalpiciclib plus cetuximab. The results showed no significant correlations between these baseline immune parameters and

response, PFS, or OS.

We fully agree that assessing dynamic changes in immune infiltration would be critical to validate the aforementioned observational phenomenon. Therefore, in our ongoing randomized controlled trial (“Dalpiciclib Combined with Cetuximab versus Cetuximab Alone in Patients with HPV-Negative, Anti-PD-1-Resistant Recurrent or Metastatic Head and Neck Squamous Cell Carcinoma: An Open-Label, Randomized Controlled, Phase II Trial”, NCT06935188), we have proactively implemented protocols to collect both pre- and post-treatment blood and tissue samples. These samples will be used for single-cell sequencing and related assays to systematically characterize changes in immune cell infiltration. Once again, we sincerely appreciate your valuable suggestion, which has provided important new directions and insights for our subsequent research.

3. Clearer discussion of the limitations and the exploratory nature of biomarker findings:

• The authors should highlight the exploratory nature and potential significance of findings such as KCNMA1, TNXB, and TPCN2 alterations in nonresponders. While these genes may plausibly contribute to resistance mechanisms through known signaling pathways, their roles as biomarkers in HNSCC or in the context of CDK4/6 inhibitor therapy are not established in the current literature.

• Please also discuss limitations such as the small biomarker cohort, lack of functional validation, and the need for independent confirmation.

Re: Thank you so much for your valuable advice. In our study, KCNMA1, TNXB, and TPCN2 alterations were significantly higher in nonresponders, suggesting to be acted as potential biomarkers in dalpiciclib resistance. Additionally, PI3K/Akt pathway is subsequentially activated by these alterations, activation of PI3K/Akt sustained CDK2/Cyclin E2 expression, rendering the cells able to bypass CDK4/6 pathway, inducing resistance to CDK4/6i.[1] Prior studies observed promising objective response rate in patients with hormone receptor-positive, HER2-negative advanced breast cancer receiving PI3Ki and CDK4/6i combination therapy.[2,3] Our observations indicated PI3Ki with CDK4/6i combination therapy

with might achieve better efficacy in patients with R/M HNSCC.

For biomarker analysis, biomarkers findings such as KCNMA1, TNXB, and TPCN2 alterations in nonresponders were analysed from a relatively small patient cohort. Downstream functional validation and the independent confirmation of these alterations are also needed in further study. We have added the limitation of biomarker analysis in the revised manuscript.

References

1. Herrera-Abreu MT, Palafox M, Asghar U, Rivas MA, Cutts RJ, Garcia-Murillas I, Pearson A, Guzman M, Rodriguez O, Grueso J, Bellet M, Cortés J, Elliott R, Pancholi S, Baselga J, Dowsett M, Martin LA, Turner NC, Serra V. Early Adaptation and Acquired Resistance to CDK4/6 Inhibition in Estrogen Receptor-Positive Breast Cancer. Cancer Res. 2016 Apr 15;76(8):2301-13.
2. Layman RM, Han HS, Rugo HS, Stringer-Reasor EM, Specht JM, Dees EC, Kabos P, Suzuki S, Mutka SC, Sullivan BF, Gorbatchevsky I, Wesolowski R. Gedatolisib in combination with palbociclib and endocrine therapy in women with hormone receptor-positive, HER2-negative advanced breast cancer: results from the dose expansion groups of an open-label, phase 1b study. Lancet Oncol. 2024 Apr;25(4):474-487.
3. Konstantinopoulos PA, Lee EK, Xiong N, Krasner C, Campos S, Kolin DL, Liu JF, Horowitz N, Wright AA, Bouberhan S, Penson RT, Yeku O, Bowes B, Needham H, Hayes M, Sawyer H, Polak M, Shea M, Cheng SC, Castro C, Matulonis UA. A Phase II, Two-Stage Study of Letrozole and Abemaciclib in Estrogen Receptor-Positive Recurrent Endometrial Cancer. J Clin Oncol. 2023 Jan 20;41(3):599-608.

4. Improved contextualization with existing literature and justification of study design choices:

• The manuscript would benefit from more direct comparison to outcomes with other regimens (e.g., cetuximab monotherapy, other CDK4/6 inhibitors), as well as a rationale for the single-arm design and chosen dosing strategy.

If these revisions are addressed, the manuscript will make a valuable contribution to the field and provide a strong foundation for future research in this area.

Re: Thank you very much for your valuable suggestion. The ORR of cetuximab monotherapy as second line treatment in patients with R/M HNSCC was only 13%, with 2.3 months of median PFS and 5.9 months median OS.[1] In the INTERLINK-1 trial, cetuximab monotherapy in HPV negative R/M HNSCC patients after failure of both platinum-based chemotherapy and previously treated with PD-1 inhibitors only had an ORR of 23.9%, median PFS and OS were 3.8 months and 8.6 months.[2] In our single-arm trial, we observed the ORR, median PFS and OS were 67.9%, 7.0 months, and 17 months with dalpiciclib plus cetuximab, indicating the promising response and survival benefit in this population. Palbociclib, another CDK4/6 inhibitor, was reported to have an ORR of 39% in platinum-resistant, HPV-negative R/M HNSCC, the median PFS and OS were 5.4 months and 9.5 months respectively.[3] In our study, we observed a better response and survival benefit in PD-1-resistant, HPV-negative R/M HNSCC by dalpiciclib plus cetuximab.

References

1. Vermorken JB, Trigo J, Hitt R, Koralewski P, Diaz-Rubio E, Rolland F, Knecht R, Amellal N, Schueler A, Baselga J. Open-label, uncontrolled, multicenter phase II study to evaluate the efficacy and toxicity of cetuximab as a single agent in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck who failed to respond to platinum-based therapy. J Clin Oncol. 2007 Jun 1;25(16):2171-7.
2. J. Fayette, L.F.L. Licitra, K.J. Harrington, R. Haddad, L.L. Siu, Y.C. Liu, M. Tahara, J-P. Machiels, D. Rischin, T. Seiwert, R. Ferris, U. Keilholz, A. Psyrri, B. Keam, P. Bossi, R. Metcalf, O. Serrano, P. Kaur, D. Ruscica, R.B. Cohen, 854O INTERLINK-1: Phase III study of cetuximab (CTX) ± monalizumab (M) in participants (pts) with recurrent/metastatic head and neck squamous cell carcinoma (R/M HNSCC) with disease progression on/after platinum chemotherapy (CT) and previously treated with an immune checkpoint inhibitor (ICI). Ann Oncol. 2023;34:S554-S555.
3. Adkins D, Ley J, Neupane P, Worden F, Sacco AG, Palka K, Grilley-Olson JE, Maggiore R, Salama NN, Trinkaus K, Van Tine BA, Steuer CE, Saba NF, Oppelt P. Palbociclib and cetuximab in platinum-resistant and in cetuximab-resistant human papillomavirus-

unrelated head and neck cancer: a multicentre, multigroup, phase 2 trial. Lancet Oncol. 2019 Sep;20(9):1295-1305.

Reviewer #2 (Remarks to the Author):

This paper explore Dalpicilib in combination with cetuximab after failure of anti-PD1. This is a small monoarm study well conducted.

We observe a clear signal of efficacy with high response rate and promising PFS and OS even if it is difficult to be confident in a mOS of 17 months with only 9.2 months of median PFS.

This drug remains toxic with 85% of patinets who need to reduce the dose: in further studies perhaps the schedule/dosage should be modified.

Re: Thank you very much for your valuable suggestion. Since CDK4/6 inhibitors have not yet been explored in patients with R/M HNSCC progressed on PD-1 inhibitors, we here designed a single-arm, small-sample study to preliminarily investigate the efficacy and safety of this regimen. Based on the potential efficacy demonstrated in this study, we are currently conducting a randomized controlled trial to further verify the efficacy of this regimen (NCT06935188). Considering the relatively high proportion of patients who experienced dose reductions due to drug toxicity, we will explore the optimal efficacy and medication schedule/dosage in the future study.

In the discussion the response rate to cetuximab alo,e after anti-PD1 is 20% in the interlink study and this level should probably be indicated. But clearly the combination alpicilib/cetuximab seems better.

Re: Thank you very much for your valuable comments. In the INTERLINK-1 trial, cetuximab monotherapy in HPV negative R/M HNSCC patients after failure of both platinum-based chemotherapy and previously treated with PD-1 inhibitors only had an ORR of 23.9%, median PFS and OS were 3.8 months and 8.6 months.[1] In our single-arm trial, we observed the ORR, median PFS and OS were 67.9%,

7.0 months, and 17 months with dalpiciclib plus cetuximab, indicating the promising response and survival benefit in this population.

References

1. J. Fayette, L.F.L. Licitra, K.J. Harrington, R. Haddad, L.L. Siu, Y.C. Liu, M. Tahara, J-P. Machiels, D. Rischin, T. Seiwert, R. Ferris, U. Keilholz, A. Psyrri, B. Keam, P. Bossi, R. Metcalf, O. Serrano, P. Kaur, D. Ruscica, R.B. Cohen, 854O INTERLINK-1: Phase III study of cetuximab (CTX) ± monalizumab (M) in participants (pts) with recurrent/metastatic head and neck squamous cell carcinoma (R/M HNSCC) with disease progression on/after platinum chemotherapy (CT) and previously treated with an immune checkpoint inhibitor (ICI). Ann Oncol. 2023;34:S554-S555.

Reviewer #3 (Remarks to the Author):

1. Although the protocol stated that the full analysis set (FAS) is to consist of "all enrolled subjects who have taken the study drug at least once", the study's analysis population apparently excludes 2 withdrawals (Figure 1B). Unless they withdrew consent, they should be included in the analyses. They can be used for OS analyses regardless.

Re: Thank you very much for your carefully review. These two patients withdrew their consents prior to the administration of study drugs, therefore, we did not include these two patients in the full analysis set (FAS), we revised the "withdrawals" to "withdrew consents" to avoid ambiguity.

2. The statement in the Introduction, "The incidence of human papillomaviruses (HPV)-negative HNSCC is higher than that of HPV-related HNSCC, which is often associated with lower response rate and poorer survival with immunotherapy.", seems reversed.

Re: Thank you very much for your carefully review. Epidemiologically, the incidence of human papillomaviruses (HPV)-negative HNSCC is higher than that of HPV-related HNSCC.[1] Mandal et al. reported in an analysis of TCGA found that HPV-positive HNSCCs had more significant immune infiltration and had

higher T cell levels infiltrated in HPV positive tissues and associated with a favorable prognosis,[2,3] which suggests patients with HPV-positive status may obtain more benefit from immune checkpoints blockade. Keynote 012 study was the first clinical trial to evaluate the relationship between HPV status and immune checkpoints blockade response. 60 patients with PD-L1^{+} (> 1%) were treated with pembrolizumab. ORR was 24% (95% CI, 13-40%) in those patients with HPV positive, and 16% in HPV negative patients (95% CI, 10-23%, P > 0.05).[4] In the Checkmate 141 subgroup analysis, patients with p16 positive tumors tend to obtain more benefit from nivolumab than the standard treatment group (median OS: 9.1 months vs. 4.4 months HR: 0.56; 95% CI: 0.32 to 0.99). On the other hand, in patients with p16 negative disease, the median OS was 7.5 months in the nivolumab monotherapy group and 5.8 months in the standard treatment group (HR: 0.73; 95% CI: 0.42 to 1.25).[5] These results indicate that HPV positive HNSCC is often associated with higher response rate and better survival with immunotherapy. To avoid ambiguity, we changed our statement from "The incidence of human papillomaviruses (HPV)-negative HNSCC is higher than that of HPV-related HNSCC, which is often associated with lower response rate and poorer survival with immunotherapy." to "The incidence of human papillomaviruses (HPV)-negative HNSCC is higher than that of HPV-related HNSCC. However, HPV-negative HNSCC is often associated with lower response rate and poorer survival with immunotherapy compared with that of HPV-related HNSCC."

References

1. Sun Y, Wang Z, Qiu S, Wang R. Therapeutic strategies of different HPV status in Head and Neck Squamous Cell Carcinoma. Int J Biol Sci. 2021 Mar 10;17(4):1104-1118.
2. Sridharan V, Margalit DN, Lynch SA, Severgnini M, Zhou J, Chau NG. et al. Definitive chemoradiation alters the immunologic landscape and immune checkpoints in head and neck cancer. Br J Cancer. 2016;115:252–60.
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of Viral- and Carcinogen-Driven Head and Neck Cancer. Immunity. 2020;52:183–99.

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5. Harrington KJ, Ferris RL, Blumenschein G Jr, Colevas AD, Fayette J, Licitra L. et al. Nivolumab versus standard, single-agent therapy of investigator's choice in recurrent or metastatic squamous cell carcinoma of the head and neck (CheckMate 141): health-related quality-of-life results from a randomised, phase 3 trial. Lancet Oncol. 2017;18:1104 – 15.

3. The 95% confidence interval for PFS extends down to 0. This seems unlikely and should be checked.

Re: Thank you very much for your carefully review. We checked our PFS raw data and found an error occurred when running the analysis in SPSS. The correct median PFS was 7.0 months (95% CI, 4.13-NR). The reason for the missing upper limit is that a relatively high proportion of patients had no tumor progression and the survival curve has not declined completely. Therefore, the maximum possible value of the median progression-free survival (PFS) time cannot be determined.

4. The legend of Fig. 4A) should include descriptions of the two marginal bar charts. Congratulations on these important results.

Re: Thank you very much for your carefully review. We have added descriptions of the two marginal bar charts. The bar chart on the right of the percentages represented the count of patients with mutations. The bar chart on the right of the mutation counts of patients represented the types of gene alternation. Bottom bars show responders (marked as blue, complete or partial response [n = 12]) and non-responders (marked as red, stable or progressive disease [n = 8]).

Here, we would like to thank all the reviewers for your help to improve the quality of our manuscript.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have addressed the previous concerns thoughtfully, and the noteworthy results are clearly presented. The work holds significance for the field and is well positioned in relation to the established literature. The conclusions are well supported by the data, and the methodology meets the expected standards, with sufficient detail to allow reproducibility.

There are no major flaws in the data analysis or interpretation that would prevent publication. Any questions/ suggestions previously raised have been adequately addressed.

In summary, I find the manuscript suitable for publication.

Re: Thank you so much for your valuable advice. All of your comments are valuable and helpful in revising and improving our paper, as well as providing important guidance for our research.

Reviewer #2 (Remarks to the Author):

OK now

Re: Thank you very much for your valuable comments.

Here, we would like to thank all the reviewers for your help to improve the quality of our manuscript.