Author's response to reviews

Title: Copy Number Amplification of the PIK3CA Gene Is Associated with Poor Prognosis in Non-lymph node metastatic Head and Neck Squamous Cell Carcinoma

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Author's response to reviews: see over
Thank you for your response to our manuscript, “Copy Number Amplification of the PIK3CA Gene Is Associated with Poor Prognosis in Non-lymph node metastatic Head and Neck Squamous Cell Carcinom”, which we submitted to BMC Cancer.

We provide point-by-point response to each of the reviewers' comments.

Reviewer #1: Statistical analysis:

1. Do the author mean adj for mutation status of KRAS and PIK3CA or PIK3CA only or KRAS only?
Response; Cox proportional hazard models were fitted for multivariate analysis adjusting for mutation status of KRAS and PIK3CA. We corrected page 8, 4th lines.

Page 8, first 3 lines, look repetitive.
Response; It is according to your indication. So we corrected page 8, 6th line.

Page 8, bottom line: "... one patient had a high score, ..." please define high score in text. The readers are not expected to find the definition themselves.
Response; We defined a high score of smoking status meant more than 30 pack-years. We added comment page 9 3rd lines.

Discussion:
Page 11, line 12: "In these little frequency..." Do not understand...
Do these mutations/amplifications reflect the exposures?
Response; We wanted to say that our small frequency of the KRAS, BRAF and PIK3CA mutation may be caused by the fact that a specimen was limited with 115. So we corrected the sentence.

Tables:
Table 6: please report p-value instead of "not significant"
How many cases with amplified PIK3CA had PIK3CA and KRAS mutation?
Response; We added p-value in table 6. And we added sentence about mutation cases at page 9, line13.
Reviewer#2;
Major Compulsory Revisions
Methods
1. It is still not clear if the recruitment was done occasionally or with respect to all patients who consecutively were submitted to surgical intervention. Furthermore, were there patients denying to be enrolled? Which are their characteristics? Response; 115 specimens were consecutively submitted by surgical management. And there are no patients who deny to be enrolled. We added sentence page 5, 5th lines bottom.

2. The description of time at risk is still weak: when did the Authors start to count it? Which kind of assessment was done to establish the relapse? Who did perform them? Response; We added sentence about follow up and relapse page 7, 6th lines bottom.

3. Why did not the Authors take into consideration further variables in multivariable approach (i.e. gender, length of the disease, etc). Response; We had examined in consideration of gender, but fell out to write it in a sentence. And we thought the length of the disease were not important for this analysis.

4. The Authors did add relative frequencies in Table 6 but they seem nonsense (i.e. primary site: percentages are neither row nor column percentages). Furthermore, some percentages are still missing. I do not contest the application of Mann Whitney test, but it should be referenced in methods section. I still believe that in same cases the Fisher exact should have been necessary. Response; We added corrected relative frequencies in Table 6. And we conducted examination about lymph node metastases with Fisher test and obtained similar results.

5. The percentage of disease free survivors at 2 years should be discussed carefully; I did ask the Authors to deal with informative censoring but no detail was provided. Response Actually 19 patients had a recurrence in PIK3CA amplified patients
and 30 patients had a recurrence in not amplified patients.

6. Did the Authors perform the multivariable approach also for overall survival? Response; We performed the same approach for over-all survival and there was no significant association. We corrected sentence at page 11,7th line.

Discussion

7. The limits of the study are not discussed at all. Response; We added sentence about the limitation at page 15,1st line.

Minor Compulsory Revisions

Methods
8. There is a duplicate sentence at the end of the part on statistics. Response; We corrected page 8,5th line.

Tables
9. The Authors should check rounding in tables (i.e. table 5). Response; We checked in tables.

Discussion
10. Discussion does still present problems with rounding of percentage (KRAS is not 3% but 2,6%; with respect to reference 11 the percentage of patient with mutations should be 10,5% instead of 11%; PIK3CA is present in 32,2% of patients and not in 31,1% and this is less than one third - no more -). Response; We corrected each point in Discussion ,page 13.