Reviewer's report

Title: Copy Number Amplification of the PIK3CA Gene Is Associated with Poor Prognosis in Head and Neck Squamous Cell Carcinoma Prospective Cohort Study

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Reviewer: Chiara de Wauré

Reviewer's report:

The paper “Copy Number Amplification of the PIK3CA Gene Is Associated with Poor Prognosis in Head and Neck Squamous Cell Carcinoma: Prospective Cohort Study” deals with the frequency and the clinical implications of mutations and amplification of KRAS, BRAF and PIK3CA in HNSCC. The topic is quite interesting but I believe that the paper cannot be suitable for publication unless very important and deep major revisions. Furthermore, in my opinion, the title of the article does not seem to be appropriate because of the constraints of the survival analysis and the disagreement with the objective the Authors wanted to pursue.

Major Compulsory Revisions

Abstract
1. I would like to ask the Authors to check the sentence “Kaplan-Meier survival analysis revealed that copy-number amplification of PIK3CA was markedly associated with cancer relapse in patients without lymph node metastasis (Log-rank test, p=0.447 and p=0.2626)” and “Copy number amplification of the PIK3CA gene is associated with poor prognosis in HNSCC patients without lymph node metastasis” because, according to the statistical tests which were reported, there is no evidence of significant association.

Introduction
2. I would suggest the Authors to better define the objective of the study with respect to type of mutations which were under investigation

Methods
3. Were cases consecutively enrolled among patients admitted to the surgical department or was there a source of selection bias?
4. The description of follow up of patients as well as of outcomes (in particular survival) and covariates is completely missing.
5. The description of statistical analysis is poor. The outcome of the study should be to analyze the prevalence of mutations and amplifications and to describe if there are dependent on patients’ characteristics. Furthermore, mutations as well as amplifications and other covariates should be put in relation with survival. In
this case, I would suggest a multivariable approach too (at least for PIK3CA because of the higher number of patients with amplification).

Results
6. In table 3 I would add relative frequencies for all qualitative variables.
7. The percentage of patients with mutation in KRAS exon 1 is 5.2% and not 6%.
8. The Authors stated to investigate the correlations between the presence of mutations and the clinical data: this was not described in the methods section and was not dealt with thoroughly in results. The only data which were provided were those about cancer site and the smoking status (which, furthermore, was not reported in the Table 3).
9. The numbers of patients with amplifications are not the same of the abstract!!
10. Table 6 could be implemented adding relative frequencies. Furthermore, the Authors did not speak about the application of the Mann Whitney test: they should specify why they chose it. According to me if the variable “number of lymph nodes sites” is categorized, the Chi square test could be applied. Furthermore, I suppose that in same cases the Fisher exact should have been necessary because of the presence of cells with an expected count less than 5.
11. The Authors did not specify anything about the follow up study in the methods and in results. They speak about overall survival (never cited before) and disease free survival without any previous clarification.
12. Why the Kaplan-Meier curves and the log rank test were used only for investigating the role of the status of PIK3CA? I would suggest studying also KRAS even though only 10 (or 12 as in the abstract??) showed the amplification.
13. Figure 1 does report only disease free survival. The title is moreover misleading.
14. The sentence “Of those with PIK3CA copy number alteration, 31% were disease-free at 2.0 years, whereas 90% of the patients without PIK3CA copy number gain survived without recurrence during the study period” is misleading because Authors did not address censoring. Did someone die being an informative censoring? Furthermore, I would suggest reporting median time to progression.

Discussion
15. The limits of the study are not discussed at all.

Conclusion
16. Conclusion must be checked because mostly in contrast with the content of paper (see also the comments on the abstract).

Minor Compulsory Revisions
Abstract
17. Please revise the percentages in the abstract: 32.1% should be rounded to 32.2% and 19.3% is indeed 19.1%.
Methods
18. Please provide the reference for the “6th UICC TNM classification and stage groupings”.

Results
19. The results of mutation in KRAS exons 2 are not reported in results even though it was said (in methods) that they were searched.
20. Please check the 32.1% percentage because it should be rounded to 32.2%

Discussion
21. I would suggest the Authors to report the right percentages: 2.6% showed mutations in KRAS gene, and 2.6% in PIK3CA.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests