Response to decision letter for manuscript 44742 entitled "FGFR4 Single Nucleotide Polymorphism Gly388Arg in Head and Neck Carcinomas"

We are grateful for the reviewer's valuable comments and have taken all suggestions seriously. Reviewer's critiques are addressed section by section in this document, and corrections were incorporated in the manuscript accordingly. All changes in the manuscript are highlighted for better traceability.

Reviewer's comments:

Reviewer #1:

No specific comments to the authors

Author's answer: We gratefully acknowledge the reviewer's assessment.

Reviewer #2:

My pleasure to review the manuscript about the clinical analysis of FGFR4 SNP in head and neck cancer by Wimmer et al. This is a careful study in the field. I have the following comments.

Table 1, Table 3 and Table 4, please remove the column 'Remarks,' that is a redundant expression since P-value has the whole story.

Author's answer: We thank the reviewer for this advice. We changed the respective table accordingly.

Table 5, logistic regression, please show the result of odds ratio and corresponding 95% CI.

Author's answer: We included results of odds ratio in Table 5.

Page 6, Line 4, misspelled word: separated

Author's answer:
We corrected the word "separated"

In Methods, Immunohistochemistry: The authors should clearly state that what protein is the target for the IHC staining in this paragraph.

Author's answer:
We changed this paragraph and name the protein, which was targeted in IHC staining.

Table 3. The details methods and criteria to distinguish low from high expression of FGFR4 should be also explicitly and stated in the Methods section.

Author's answer:
We changed this section accordingly.

In the Discussion, the authors jump-started to discuss angiogenesis in the head and neck cancer. However, what all about the angiogenesis is related or linked to FGFR4 were not touched upon and well-referenced. This part should be re-written.

Author's answer:
We thank the reviewer for this advice. We now extended the Discussion section accordingly and also included a scheme.

Reviewer #3:
The study addressed the potential clinical significance of FGFR4 Arg388 (single-nucleotide polymorphism) in head and neck carcinoma. The authors tested 284 patient samples using immunohistochemistry and PCR. The tested number is quite large, thus, supporting the significance of findings. The data indicates that advanced tumor stage and local metastasis are significantly associated with reduced disease-free survival in mutant FGFR4 Arg388 carriers (patients). Furthermore, increased expression of FGFR4 correlated significantly with worse overall survival in the tested

cancer patients. The authors suggested that FGFR4 Arg388 represents a useful target for future multimodal therapeutic interventions. The study is an interesting and well-written. There are several minor questions to address.

1. It is necessary to indicate (Methods section) whether the study was approved by a relevant Human Ethics committee.

Author's answer: We included a statement on study approval by the ethics committee in the method section.

2. Immunohistochemistry (IHC) section, page 5: "…incubated with 1:100 pre-diluted primary antibodies (clone 16; Santa Cruz,…" it is necessary to add "FGFR" before primary abs, and if possible it is necessary to include cat# for the used abs.

Author's answer: We changed this section accordingly. Unfortunately, this clone has been discontinued in the meanwhile by the company and replace by another clone. Therefore we cannot provide the catalogue number. We apologize. https://www.scbt.com/scbt/product/fgfr-4-antibody-c-16

3. Authors wrote; "Staining intensity was differentiated into three groups: 1. + (weak), 2. ++ (moderate), and 3. +++ (high)." It is necessary to include exemplary photographs to illustrate each group. Fig.2 is supposed to present all categories, however the 'moderate" category is missing. The "low" category looks not so very low.

Author's answer: We now rearranged Figure 2 and included examples of all categories of FGFR4 expression levels.

4. How the categorization was done? It is unclear. Was it manually categorized/ how many people were involved into categorization? Was it done blindly (the researchers

were not supposed to know the patient's name, conditions etc). The methods section should include more detailed description of IHC as it is one of the main parts of the study.

Author's answer:
We thank the reviewer for this important remark. We now included a more detailed description about IHC and assessment of receptor expression.

5. Reference section can be improved and should include the following papers: Choi KY, Rho YS, Kwon KH, Chung EJ, Kim JH, Park IS, Lee DJ. ECRG1 and FGFR4 single nucleotide polymorphism as predictive factors for nodal metastasis in oral squamous cell carcinoma. Cancer Biomark. 2012-2013;12(3):115-24. doi:10.3233/CBM-130299. Gao L, Lang L, Zhao X, Shay C, Shull AY, Teng Y. FGF19 amplification reveals an oncogenic dependency upon autocrine FGF19/FGFR4 signaling in head and neck squamous cell carcinoma. Oncogene. 2018 Dec 5. doi: 10.1038/s41388-018-0591-7.

Author's answer:
The references were added to the manuscript and included into the discussion.

6. Discussion section should be also extended and describe potential mechanisms of FGFR4 signaling (shortly, anti-apoptotic , pro-angiogenic effects etc)/ the authors might include a schematic presentation of FGFR4 signaling in head in neck cancers.

Author's answer:
We thank the reviewer for this advice. We now extended the Discussion section accordingly and also included the requested scheme.

We thank the reviewers for their exceptionally helpful and constructive suggestions and hope that the revised manuscript is now acceptable for publication.