Author's response to reviews

Title: The human epidermal growth factor receptor (EGFR) gene in European patients with advanced colorectal cancer harbors infrequent mutations in its tyrosine kinase domain

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Author's response to reviews: see over
Dear Editor,

Please find our revised manuscript entitled “The human epidermal growth factor receptor (EGFR) gene in European patients with advanced colorectal cancer harbors infrequent mutations in its tyrosine kinase domain”, MS: 4960196175557456.

- All the changes made are highlight in red (using the tracking system of Microsoft word) except when the entire paragraphs have been moved (for example, “methods” is now between background and results).
- This manuscript has been corrected for the English.
- This manuscript has been completely reformatted to fit the recommendation of your editorial policy.
- The title page has now the full detailed address of each authors as well as email address for each of us.
- Abstract is now subdivided in four parts (background, methods, results and conclusions).
- “Methods” have been moved from after previous “Results/Discussion” to between Background and Results.
- Previous results/discussion paragraph is now divided in two separate paragraphs, as suggested by referee number 1 and the editorial policy.
- A “Competing interests” paragraph have been include after the “Conclusions”.
- The Figure has been deleted, only remain the legend.
- Tables have been completely rebuild using word tool in order to delete vertical lines and titles are now above the tables whereas legends still remain under them.

Referees comments have also been addressed:

Referee num 1

1. From the previous literature it is well known that the frequency of these mutations is very low. The relevance of the study is consequently questionable, and the reasons for performing the analysis and investigating these mutations are not obvious.
   - As mentioned in the background paragraph, this study has been engaged as there is only one study focusing on European patients and moreover on a very small number of patients. As difference in EGFR mutation frequency seems to exist between continents (for example Japanese patients harbour a higher mutation frequency in their EGFR gene than in United States) as mentioned in the same paragraph, it seemed pertinent to undertake this study, especially since EGFR in the target of new therapy. Nevertheless, to clarify the interest of our study we add this sentence in the introduction: “We studied a large cohort of a European population in order to investigate the frequency of occurrence of mutations in the EGFR gene in a European context and to determine if these mutations occur as frequently as in American population”.

2. In the background section the authors state that the EGFR mutations are responsible for resistance to EGFR in general, and the role of KRAS mutations is not mentioned. It is important to distinguish between the monoclonal antibodies which have show efficacy in CRC and TKI which have not. This paragraph should therefore be rewritten.
   - The paragraph has been modified.
3. The KRAS mutations are responsible for the majority of resistance to the EGFR MoAbs, and a relation between the KRAS status and small mutations would therefore be relevant.

- K-ras mutation data has been added in table 2 and are discussed in the discussion paragraph.

4. The impact of the data here presented is unfortunately limited as also acknowledged by the authors. The sample size is too small to allow for an investigation of the association between mutational status and the clinical parameters which would be of interest. Furthermore, the functional importance of the mutations e.g effect on gene expression levels/ protein levels, responsiveness to EGFR inhibition etc. has not been mentioned in the discussion. The data would be of interest if a functional importance of these mutations was presented, but a purely descriptive report of low frequency mutations has limited impact.

- The main objective of this work was to test the hypothesis that mutation in EGFR gene may explain the failure of the use of anti-EGFR therapy in colorectal cancer. The description of new mutations was unexpected and could be taken as a plus for our results. The functional experiments suggested by the referee are indeed promising and interesting and would be the next step of our research. However, given the complexity and time required by this type of approach, these ideas are now mentioned in the discussion as an open perspective.

5. The combined result and discussion section should be separated into two independent sections to allow for a relevant discussion of the value of the data, and the limitations of the study which have not been mentioned.

- A discussion paragraph has been created and discussion about k-ras status and weakness of our results presented.

Referee num 2

1. Throughout the text authors do not discriminate between mutations and SNPs found in EGFR of CRC patients. Example: silent germinal point mutation of exon18 (Y725Y), exon19 (742), exon20 (787), exon21 (836).

- The text has been corrected according to the referee recommendation. SNPs are discriminate and a paragraph about them cold be found in the discussion part.

2. Definition of the amplification of EGFR is completely based on MLPA technique used. The reviewer does not feel correct this definition: a maximum what can be accepted is the term copy number increase including polysomy. Other problem with the definition is that how authors interpret those data when there is a discrepancy of EGFR levels in various exons as the MLPA kit offers. Why a minimum threshold of 5 exons is defined for copy number alteration? Suggestion is to delete the amplification part of the entire manuscript.

- Although these results support the sampling of our cohort, we remove all the texts referring to the MLPA technique as requested by the referee.
We hope that these changes will convince you to publish our article in your journal we wish you good reception.

Best greetings

Thomas Wenner Ph. D.