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Editor Dr Ze-Mao Gong

Editorial Office

World Journal of Gastroenterology

Response Letter ESPS manuscript NO:32886

Dear Editor,

We are submitting our revised version of the manuscript (ESPS Manuscript NO. 32886) titled "**Characterizing gastrointestinal stromal tumors and evaluating neoadjuvant imatinib by sequencing of EUS-biopsies**" by Hedenström Per, Nilsson Bengt, Demir Akif, Andersson Carola, Enlund Fredrik, Nilsson Ola, and Sadik Riadh for publication in World Journal of Gastroenterology as a Clinical Trial Study.

We would like to thank the Reviewers and Editors for the constructive comments upon our manuscript. We have carefully addressed these points and the manuscript text has been revised as presented in this new version we now re-submit for Your kind evaluation. All changes in the manuscript are highlighted by blue colored words.

A detailed response to the reviewers is provided below (page 2).

According to the Editor´s instructions we have retyped the reference numbers in the appropriate style and we have moved all tables and figures from within the manuscript text to the space after the reference list. We have also updated all the required documents enclosed together with the manuscript according to Your instructions (*Guidelines and Requirements for Manuscript Revision-Clinical Trials Study*, No. 1-14).

We hope that that this revised version of the manuscript is now acceptable for publication in *World Journal of Gastroenterology*.

Sincerely Yours

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Detailed response to the reviewers:

Reviewer 02941552:

1. Imatinib therapy is usually needed in high risk GIST. Is it needed neoadjuvant therapy by imatinib in all cases? The size of your cases in table 1 shows minimum size of only 12 or 13 mm.

As the reviewer correctly comments, preoperative neoadjuvant imatinib therapy is not warranted in all cases diagnosed with a GIST. We have clarified this issue in the methods section of the revised manuscript (marked in blue). In general, small size GISTs (< 2 cm) are not high risk tumors and these tumors were not evaluated for or subjected to preoperative imatinib treatment in the present study. In our study, and according to Table 3, the cases denoted *Neo-* (n=12) were not treated with neoadjuvant imatinib but only the cases denoted *Neo+s* (n=10) and *Neo+r* (n=5).

2. Why did you divide the period 1 and 2. I think that it might be more clear to design this study like period 2 from the beginning.

We appreciate this comment upon the study design and the nomenclature of the two study periods. The study cohort named *Period 2* in the original version of the manuscript has now been retitled the *Study Period* (SP) – marked in blue. Moreover, the study cohort named *Period 1* in the original version of the manuscript has now been retitled the *Baseline Period* (BP) – marked in blue.

During the *Study Period* the study design was interventional, i.e. all study cases were subjected to dual sampling with both EUS-FNA and EUS-FNB in a randomized order. During the *Baseline Period* the study design was not interventional but observational, i.e. the cases sampled in 2006-2011 were punctured according to the discretion of the attending endosonographer and according to clinical practice (mainly single EUS-FNA). That is the reason why the sampling approach is not alike in cases punctured 2006-2011 and in cases punctured 2012-2015.

The main reason to include the GIST-cases of the *Baseline Period* in the manuscript is to show to the increase in diagnostic sensitivity obtained by changing from the routine EUS-guided sampling procedure previously used in our center to the new sampling approach using EUS-FNB (the *Study Period*). A second reason is to demonstrate that the dual sampling procedure used in the *Study Period* (EUS-FNA and EUS-FNB in the same lesion) did not have any negative impact upon the sensitivity of EUS-FNA, which was the main sampling approach in the *Baseline Period*.

3. In table 3, What sort of arrangement did you use for the case numbers of Table 3. I think it is better to arrange the patients by similar group.

We agree to the reviewer´s comment that the order of the cases in Table 3 was not clearly specified in the original version of the manuscript and that the cases can be arranged in different ways.

We have highlighted the method used to order the cases in the legend of Table 3 (marked in blue). The order of these 44 cases was based on the date of the study subject enrollment; i.e. case # 1 was the first case included in the study, case #2 was the second case included in the study and so on. We believe that ordering the cases by chronology properly describes the study inclusion progress and is neutral with respect to the data. The arrangement of cases in Table 3 based on prognostic risk would be challenging since a prognostic risk assessment according to the WHO consensus criteria is invalid in cases treated with neoadjuvant imatinib. Therefore we have kept the same order of arranging the cases of Table 3 in this revised version of the manuscript now re-submitted to the Editorial office. However, the study cases have indeed been grouped with respect to the preoperative management (*Neo-*, *Neo+s*, *Neo+r*). This information is shown in Table 3 and depicted in Figure 3A-C.

We hope that this way of presenting the data is beneficial for the readers of the manuscript and demonstrates the impact of imatinib on the Ki-67 index, which is a key message of the article.

4. Supplementary data is only table 3. Where is the supplementary methods and supplementary table 1?

We are thankful for the reviewer's request on clarification of the supplementary material.

In the revised manuscript now re-submitted to the Editorial Office we have included Table 3 in the body of the manuscript and not as a supplementary file (table heading marked in blue). In the supplementary file *Supplementary Methods* You will now find both the Cytopathology and Histopathology Method and a table on the designation of cases based on the preoperative management (entitled Supplementary Table 1 in the original version of the manuscript).

Reviewer 03666824:

1. What are the inclusion criteria for suspicious patients? I think it is better to set the standard.

We are grateful that the reviewer noticed that this phrase of the original manuscript needs some clarification. We have now pinpointed in the text in the section *Methods* (marked in blue) what were the criteria for being eligible as a study subject suspicious of having a GIST

2. Why are there two stages to experiment? Why use EUS-FNA only in the first stage? I think it is better to design the first stage like the second stage.

We appreciate this comment upon the study design and upon the sampling approach of the stages.

To avoid any risk of misinterpretation, the study cohort named *Period 2* in the original version of the manuscript has now been retitled the *Study Period* (SP) in the revised manuscript. Moreover, the study cohort named *Period 1* in the original version of the manuscript has now been retitled the *Baseline Period* (BP).

During the *Study Period*, 2012-2015, the study design was interventional, i.e. all study cases were subjected to dual sampling with both EUS-FNA and EUS-FNB in a randomized order. During the *Baseline Period* the study design was not interventional but observational, i.e. the cases sampled

in 2006-2011 were punctured according to the discretion of the attending endosonographer and according to clinical practice (mainly single EUS-FNA). That is the reason why the sampling approach is not alike in cases punctured 2006-2011 and in cases punctured 2012-2015.

Despite the use of different sampling approaches in the two periods, we believe there is important information to be extracted from the comparison of the *Study Period* and the *Baseline Period*.

The first and main reason to include the GIST-cases of the *Baseline Period* in the manuscript is to show the increase in diagnostic sensitivity obtained by changing from the routine EUS-guided sampling procedure previously used in our center (mainly single EUS-FNA) to the new sampling approach using a reverse bevel FNB-needle. A second reason is to demonstrate that the dual sampling procedure used in the *Study Period* (EUS-FNA and EUS-FNB in the same lesion) did not have any obvious negative impact upon the sensitivity of EUS-FNA; being the main sampling approach in the *Baseline Period*.

3. Imatinib is expensive and has a lot of side effects, and is there still a need for neoadjuvant therapy when the lesion is completely removed? I think it is better to classify the risk grade of stromal tumors, and the tumor with low or lower recurrence risk may not be treated with neoadjuvant therapy.

We agree to the reviewer's comment that imatinib has potential side-effects and that not all GISTs should be treated with neither preoperative (neoadjuvant) imatinib. In line with this comment we have added a phrase in the methods section (marked in blue) and one phrase in the discussion (marked in blue). In these two phrases we stress that a) small size GISTs (< 2 cm) are unlikely to be high risk tumors and that these tumors were not evaluated for neoadjuvant imatinib and b) the decision on neoadjuvant imatinib therapy must be carefully evaluated in each single case based on the information available concerning prognostic risk. The side-effects of imatinib are also one of the reasons why we conducted the present study with sequencing of *KIT* and *PDGFRA* in preoperative FNB-biopsies.

We also agree to the comment that prognostic risk is an important factor in the decision on neoadjuvant therapy and that the initiation of neoadjuvant imatinib therapy, which was not a focus of this study, may vary between institutions. We have added a comment regarding this topic in the discussion (marked in blue).

In the preoperative phase of GIST-management the assessment of prognostic risk in individual cases is challenging since the information on the mitotic rate is not available. Based upon our experience, and a reason for imatinib treatment also in cases being candidates for R0-resection, neoadjuvant therapy facilitates the surgical procedure and minimizes the complication rate.