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Münster, March 14th, 2017

Re:
World Journal of Gastroenterology, Basic Study
ESPS Manuscript NO. 32261: Detection and Characterization of Murine Colitis and Carcinogenesis by Molecularly Targeted Contrast-enhanced Ultrasound

Dear Editor, dear Reviewers,

Thank you for assessing and considering our manuscript for your journal. Please find below our point-by-point response to your remarks. We appreciate your fair and respectful comments and feel that the revisions significantly improved the quality of the manuscript.

We are looking forward hearing from you.

Sincerely,

Dominik Bettenworth and Markus Brückner

For the authors

Rebuttal

Title: Detection and Characterization of Murine Colitis and Carcinogenesis by Molecularly Targeted Contrast-enhanced Ultrasound
ESPS Manuscript NO: 32261
Authors: Markus Brückner, Jan Heidemann, Tobias M Nowacki, Friederike Cordes, Jörg Stypmann, Philipp Lenz, Faekah Gohar, Andreas Lügering, Dominik Bettenworth

Step 1. Please revise your manuscript according to the reviewers' comments.

Reviewers' comment:

Reviewer 1
Comment:
The manuscript is well written, has decent scientific value. It only requires some very minor language polishing.

Answer:
We thank the reviewer for the consideration of our manuscript. *Our co-author Feakah Gohar, who is a native speaker located in Great Britain, has double-checked and revised the manuscript in order to improve the native language of the text.*

Reviewer 2
I would like to congratulate the authors for their valuable study. The study was designed very well. But I would like to suggest few improvements. You can find my recommendations below.

Comment #1:
Abbreviations should be reviewed and rewrite. Because in the beginning of the manuscript some abbreviations did not explained. Eg: DSS or AOM; page 3 line 71 and 75.

Answer:
We thank the reviewer for this relevant advice. All abbreviations were reviewed throughout the manuscript.

Comment #2:
Statistical informations could have been given more detailed in the result part of the abstract.

Answer:
More detailed information about the statistical results were included in the revised version of the manuscript (page 4, lines 114-129).

Comment #3:
Introduction part could be shortened by removing similar subjects detailed in the discussion part.

Answer:
Thank you for this important note. The introduction part was shortened accordingly.

Comment #4:
Conclusion part should be separated from the discussion part.

Answer:

Conclusion and discussion are now separated in the revised version of the manuscript (page 18, lines 876-878).

Reviewer 3

The manuscript submitted by Bruckner et al assessed a new non-invasive technique to assess severity of inflammation via MAdCAM-1 targeted contrast enhanced ultrasound (CEUS) and tumor formation using VEGF targeted CEUS. These experiments nicely correlated the histology and weight loss associated with the induction of DSS induced colitis and multiple rounds of DSS to induce carcinogenesis, to the ultrasound images captured with contrast in addition to VEGF or MAdCAM-1. This specific and non-invasive technique is sensitive to the development of inflammation and carcinogenesis and seems to be an advantageous alternative to biopsy sampling in patients. Also advantageous for investigators to utilize less numbers of mice in vivo since disease progression can be monitored without having to euthanize the animals. My only criticisms would be to

Comment #1:

Enhance the resolution and size of the histology photos for clarity.

Answer:

We thank the reviewer for bringing this to our attention. The resolution and size of the histology photos were enhanced for more clarity (figures 1, 3 and 4).

Comment #2:

Follow up on the long-term effects of binding up receptors important for the recruitment of T cells to manage the progression of inflammation. It is not clear if an additional destruction sequence is performed with ultrasound pulses to destroy the targeted antibody or what the long term consequence of this interaction may be.

Answer:

We appreciate this thoughtful remark of the reviewer.

The use of a destruction sequence for microbubbles during CEUS examination is a crucial part of this diagnostic modality and is also applied during clinically examination of human patients when administering contrast agents such as SonoVue. Given the significance of aspect, we integrated a detailed technical explanation of the destruction-replenishment sequence in the method section of the revised manuscript as follows (pages 10-11, lines 492-515):

"Importantly, the used destruction impulse destroyed only the MBs and did not affect the antibodies. Before initiating the destruction-replenishment sequence, measured echogenicity consisted of MBs bound to their endothelial target *via* antibody plus MBs flowing by in vessels. The applied destruction impulse destroyed all MBs in the field of view with the antibodies staying attached to their endothelial target and continually blocking it. After the destruction impulse, the measured echogenicity consisted only of MBs flowing by in vessels, as antibodies still blocked the endothelial targets preventing new compound from binding to the endothelial target. The difference between echogenicity after destruction sequence and echogenicity before the sequence led to values of echogenicity arising from highly specifically targeted MBs:

Echogenicity [molecularly targeted MBs + flowing MBs] - echogenicity [flowing MBs] = echogenicity of molecularly targeted MBs

This sequence enabled highly specific molecularly targeted CEUS, which required two sequences of application of contrast agent. First, a sequence with MBs bound to the particular isotype control antibody was recorded. Second, a sequence with MBs bound to specific targeted antibody (MAdCAM-1 and VEGF) was recorded."

Additionally, we underlined the therapeutic value of monoclonal antibodies specifically targeting MAdCAM as a gut-selective adhesion molecule in the discussion part of the revised manuscript (pages 17-18, lines 819-876):

"Several integrins participate in this mechanism, e.g. the interaction between the α4β7-integrin on T-cells and MAdCAM-1 addressin on endothelial cells promote the accumulation of pathogenic T-cells in the inflamed mucosa. Therapeutical blockade of T-cell homing *via* the integrin α4β7 and the cellular adhesion molecule MAdCAM-1 has been intensively studied during the last years as T cells represent a key player in the perpetuation of intestinal inflammation. Most recent work by Wendt *et al*. demonstrated that even classic glucocorticoids act partially *via* MAdCAM-1 by reducing C-C chemokine receptor type 9-mediated chemotaxis of T-lymphocytes. "(…)
"Recent research reveals differences between CD and UC with regard to the gut homing of T effector cells under treatment with vedolizumab. It could be shown that there is an unchanged homing of T effector cells in the colon of CD patients as the α4β1 expression is increased in T effector cells during vedolizumab treatment, which leads to adhesion *via* the α4β1 integrin and vascular cell adhesion molecule-1. This observation underlines that homing of T-cells proceeds *via* different and nonredundant pathways in IBD affecting treatment strategies in anti-integrin therapy and underlining the importance of monitoring long-term effects for the recruitment of T-cells very closely. Our results show that CEUS allows for direct visualization of MAdCAM-1 upregulation in inflamed areas of the bowel, which enables the detection of intestinal inflammation, visualizes the recruitment of inflammatory cells and provides an objective endpoint to assess the degree and extent of intestinal inflammation during evaluation of novel therapeutic approaches."

Editorial comments:
Don't need blank space between reference number and the before words. Please check throughout. Thank you!

Answer:
We appreciate the editor's suggestions and revised the manuscript according to his remarks. As requested, all changes were performed and highlighted in the updated version of the manuscript.