**Point-by-point letter**

“Live mini-endoscopic assessment of acute intestinal Graft-versus-Host Disease in mice”

by Vera Buchele et al., submitted together with the revised version from 10/18/2018

***Reviewer #1:***

*Manuscript Summary:*

*The study by Buchele et al. provides the detailed description of a frequently used preclinical model for graft-versus-host disease (GVHD) followed by endoscopic evaluation of intestinal inflammation. Mouse models are key for our understanding of the complex pathophysiology of GVHD and to further investigate therapeutic approaches in a preclinical setting. However, with the large number of mouse models currently available and the methodological variations amongst the GVHD community, it might be difficult to determine and successfully perform a respective approach, especially for groups that are new in the field. Furthermore, disease severity is mainly assessed by clinical scoring systems with few indicators for intestinal inflammation during disease progress, followed by histological analysis post mortem. This study describes one of the most commonly used major-mismatch mouse models, which is easy to follow and thus likely to enhance reproducibility towards the community. Further, live-assessment of colonic inflammation displays an attractive approach for groups investigating intestinal GVHD.*

*Major Concerns:*

*The methodological procedures of this study are well described. The authors further highlight the benefits of a kinetic assessment of intestinal GVHD using endoscopy. However, in this study, only endpoint endoscopic evaluations have been performed around day 30. Have the authors also tried to evaluate earlier time points during disease progression? When are the first endoscopic lesions visible? Can the authors give any recommendations about when to perform the first endoscopic evaluation after allo-HSCT? Could irradiation-induced enteritis/colitis increase the risk of perforation?*

Response to the reviewer:

We thank the reviewer for raising this important point. We sought to address these points in the revised discussion section, especially addressing the aspect of validity and sensitivity of the endoscopic approach when employed at earlier time points. According to our own published (Ullrich et al., JCI 2018) and unpublished observations, colonoscopy is suited to reproducibly and reliably detect allo-response driven colitis-induced lesions around day 15 after irradiation, i.e. 13 days after transfer of allogeneic T lymphocytes compared to noT mice receiving T cell depleted bone marrow only. At this time point routinely clinical onset of colitis can be observed. Here, endoscopic sum scores have usually returned to base line in control mice while GvHD-prone mice routinely display scores >3 with a sum score of above 3 in our experience represents a virtual threshold value indicative for the presence of colitis. However, formally we have not performed detailed correlation studies comparing endoscopic results with the gold standard histopathology. Therefore, at this point we have to refer to future studies to solidly investigate this matter to formally prove that endoscopic results are correctly staging colonic GvHD related inflammation early-on (i.e. around day 15) whereas we have shown that convincingly for the case of established colitis (i.e. around day 30) according to the data provided with this protocol.

We agree to the speculation of the reviewer that time points earlier than day 15 may be critical in respect to the specificity of the detected alterations by the endoscopic evaluation approach since irradiation induced lesions may be even more prevalent at that point and hence might dominate over the allo-T cell response-mediated GvHD-specific tissue damage. Also, as included in the revised discussion section, despite fully established intestinal GvHD perforation of the colon does not appear to represent a major adverse event in the case of proper anesthesia and careful execution of the endoscopic procedure. Hence and despite the fact that the risk of perforation during earlier time points with an assumed higher prevalence of irradiation-induced enteritis/colitis has not been in the focus of this study and has not been formally investigated, we believe that proper execution of the endoscopy as outlined in the protocol is crucial but also effective to prevent an increased occurrence of perforation events even at earlier time points. However, as pointe out above, before around day15, the added value of such an analysis is questionable at that point.

*Minor Concerns:*

*The introduction and the discussion contain the main and adequate aspects of clinical and molecular features of GVHD, that are also discussed properly. However, the authors might want to edit certain parts of the manuscript as the phrasing is sometimes redundant or sentences too lengthy (e.g. line 74-78).*

Response to the reviewer:

We thank the reviewer for his request to increase readability of our manuscript. Accordingly, we shortened and streamlined the introductions section and revised the discussion section also taking editorial comments into consideration.

**Reviewer #2:**

*Manuscript Summary:*

*An interesting detailed manuscript on mini endoscopy for assessment of murine GVHD*

*Major Concerns:*

*Please provide a more detailed analysis of endoscopic picture, histopathology and "clinical" GVHD scoring. This would significantly improve the manuscript to show how clinical grade, endoscopic grade and histopathology correlate.*

Response to the reviewer:

We thank the reviewer for raising this important point. To accommodate the reviewer`s reasonable request, we added an analysis of the relationship between endoscopic scoring and systemic GvHD levels, as depicted in the new Figure 3D. Importantly, both colonic and systemic GvHD show a high grade of accordance in line with the clinical observation that severe intestinal GvHD is often associated with a profound systemic pathology and decreased quality of life due to systemic signs of inflammation.

*Minor Concerns:*

*Introduction line 68: chronic GVHD is also mediated by donor lymphoctes*

*Introduction line 69: please add: "of the acute form of GVHD".....*

Response to the reviewer:

We thank the reviewer for pointing out these important aspects. We rephrased the indicated passages accordingly.

*Protocol: I am not sure, whether the whole transplant procedure is required in detail since most parts are published multiple times. Citation of the standard parts would be suffficient from my side. If it is the policy of the journal to provide all informations in detail I am fine with the text.*

Response to the reviewer:

As we grasped the editorial policy, the detailed description of the model is favored to accordingly follow the steps leading to the core of this manuscript, i.e. the protocol how to endoscopically assess the intestinal GvHD phenotype in live mice. Hence, we currently resisted to shorten that part of the manuscript.

*Line 388: delete "regardless"*

Response to the reviewer:

We deleted the word “regardless”.

*noT control mice: has the abbreviation been introduced?*

Response to the reviewer:

We thank the reviewer for his question. We added a more detailed explanation of this abbreviation in line 336-338 to clarify this point.