Author's response to reviews

Title: Evaluation of Immunological Escape Mechanisms in a Mouse Model of Colorectal Liver Metastases

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Author's response to reviews: see over
Dear Dr. Alam,

Again the authors would like to thank the reviewers for their qualified suggestions and annotations.

In the following we address the comments of the reviewers. The additional changes in the manuscript have been highlighted.

We would appreciate very much if you would now consider the corrected version for publication in *BMC Cancer*.

Yours sincerely

Andreas Thalheimer, M.D.
Reviewer #1:

**Minor Essential Revisions:**

1. **Abstract**
The conclusions drawn by the authors in the revised version of the abstract are not appropriate and do not reflect the main findings of the paper. The authors themselves state in their discussion that there are some major differences between the human disease and the animal model used, e.g. the degree of lymphocytic infiltration. The conclusion drawn at the end of the discussion seems more appropriate and should be used to replace the passage.

The passage in the conclusion section of the abstract has now been replaced by the conclusion drawn at the end of the discussion.

2. **Results**
*Figure 1a, 3a: Standard deviations of normal control animals are lacking and need to be presented.*

We apologize for this and have corrected the figures accordingly.

3. **Discussion**
*“Thus, the type, not the quantity of tumor infiltrating cells may be a more critical determinant for the prognosis [27].”*  
In the light of recent findings on the impact of T cell infiltration for prognosis of patients with colorectal cancer, the sentence should be rewritten and additional references referring specifically to CRC should be cited (e.g. Galon et al 2006, Laghi et al. 2009).

This is an absolute justified note which has been considered in the revised version (page 14 and 15, highlighted).
Reviewer #2:

Overall the authors have successfully improved the quality of the manuscript, sometimes even by taking over the reviewers expressions. In some cases however, they seem not to have understood all comments, several of which refer to the fact that their conclusions are inadequately supported by their data. This is specifically true for points 3 and 4 raised in my previous review.

In point 3, I criticized that counting of cells in tissues stained with antibodies against soluble cytokines is generally considered impossible among experts in tissue staining. The authors now state that their PCR data confirm their immunohistochemistry, however they don't show it. Therefore I suggest to remove Figure 1b and stay with 1a, this seems more reliable. Nevertheless, I would be interested to see in the manuscript a comment on how many normal tissues where.

Reviewer #2 is right stating that immunohistochemical staining of soluble cytokines is very difficult, yet not impossible. The re-evaluation of our immunohistochemical data shows that the expression of different cytokines could not be attributed to specific cells morphologically. Thus, the conclusion that we can demonstrate a Th1-Th2 shift of cytokines morphologically can not be confirmed by our data presented. So, according to the your suggestion, we removed all data dealing with cytokines and immunohistochemistry in the manuscript including Figure 3b (I guess, the reviewer referred to Fig 3 and not Fig 1 as written) and stayed with our RT-PCR data.

In point 4, I criticized that on the same tissue the up-regulation of FASL is shown, however not the down-regulation of FAS. Now the authors claim that they have the staining, but they don't show it. In times of supplementary material nobody can claim that one more picture would be an overload or stress for the reader. I also have the impression that the authors don't take the comments serious. So I consider it necessary that they show the staining with FAS on the same tissue sections.

I apologize for the impression that we don't take the comments serious. Quite the contrary, we are thankful since the manuscript has been improved substantially by all the criticisms and suggestions of the reviewers. We now have added the FAS-staining which is unfortunately not on the same tissue sections as the staining of FASL. Since the tissue samples were not available any more, we could not redo the staining of FAS with exact the same tissue sections. The staining shown in the corrected version of our manuscript (fig. 5b), however, is representative for the results demonstrated in figure 4b.
In point 2 I criticized that overlay with CD8 would be a CONTROL to show that a
different picture occurs as compared to the CD4/Foxp3 overlay.

I admit that I most probably did not understand this comment on your first review. However, after re-evaluation of the current literature we have changed the manuscript according to the data of the recently described T8regs in colon cancer (page 15, 16). The analyses of different subsets of regulatory T cells will be necessary in further studies on human colon cancer tissue and blood samples of tumor patients and as soon as the personal and structural situation in our lab is reconstituted we definitely will thankfully adopt this suggestion.

Minor comments:

In the discussion the authors claim that apoptotic CD8 cells increase during
the growth of metastases, again they only show one time point.

The presentation of the immunofluorescence staining at day 20 (fig 6a/b) is just a
demonstration of how this special kind of staining was performed. I hope we find your
agreement that we constrict this demonstration to just one time period since even
demonstrating every point of time following intraportal cell injection would not provide
further information in our opinion.

On page 12, line 17 the sentence is incomplete or should be reorganized

This is correct and has been changed.
Reviewer #3:

The authors have performed an intensive revision of this manuscript. Now results are well presented and discussion and conclusion are very much related to the results shown in this study.

Minor Essential Revisions

- Page 8; purchased from.
  
  Has been corrected

- Page 10, Zeiss camera (Jena) is duplicated.
  
  That is right. Has also been corrected

- Page 12; “significant higher expressed”, please rewrite.
  
  Has been rewritten

- Page 13, fig. 5c should be 5b.
  
  The figures have been revised so the numbers also have been changed. But your comment is definitely right since there has been no fig. 5c 😊

- Page 14; preclinical should be preclinically.
  
  Has been rewritten