Author’s response to reviews

Title: Semiallogenic fusions of MSI+ tumor cells and activated B cells induce MSI-specific T cell responses

Authors:

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Author’s response to reviews: see over
Dear Dr. Khong,

thank you very much for the excellent review of our manuscript MS: 3104790345241921, Semiallogenic fusions of MSI+ tumor cells and activated B cells induce MSI-specific T cell responses by Yvette Garbe, Ulrike Klier and Michael Linnebacher. We appreciate the helpful comments and modified the manuscript according to the reviewer’s suggestions. Modifications in the text of the manuscript are marked by the track-changes option of Microsoft World.

In the following are our answers on a point to point basis:

Response to reviewer 1 comments:

1. The reviewer asks for data of several fusion clones not chosen for subsequent experiments. We included the expression data of MHC and costimulatory molecules of two clones not used subsequently as asked. Additionally, she suggests displaying the flow cytometry data presented in Table 2 as primary data and not summarized in a table as we did. However, there would be now 36 primary flow cytometry plots to display. This would substantially bloat the manuscript. Since the reviewer also wants these primary data for Table 3 (additionally 22 blots) and Table 4 (additionally 14 blots), this recommendation would result in a manuscript length that would alienate potential readers. This is neither in our interests nor in the interest of the journal. Still, we agree with the reviewer that it is necessary to present some primary data. Thus, we first present some primary data as additional data for the reviewers (CD3, CD4 and CD8 of TcFc1) and second, we included another Figure into the manuscript (new Figure 4) that contains primary data that should be included as asked for in point 5 (please see there) The gating strategy for the T cells analyzed has also been included into the Material and Method section.

2. Please refer to Point 1.

3. The question raised by the reviewer is very interesting: do the Fc2 T cells perform similar than the Fc1 stimulated T cells shown in Figure 1B. Concerning the number of reacting T cells, there was no difference in the ELISpot results from Fc1 and Fc2 stimulated T cells. This is why we decided to show only one representative result. However, when re-analyzing the ELISpots, we realized that the spots of TcFc2 tend to be smaller than the ones of TcFc1. This is in line with the results of the intracellular cytokine staining. But we do not consider this to be a major result.

We additionally agree with the reviewer that the Fc-stimulated T cells have difficulties producing IFNg against HCT116. However, approximately 0.5% of the bulk culture T cells reacts against HCT116; and after the separation into CD4 and
CD8 T cells, nearly 2% of the CD8 T cells recognized HCT116 in the ELISpot assay (Figure 3B). The latter frequency is well in the range aspired for bulk T cell cultures.

4. The upregulation of MHC I expression on HCT116 when treated for 48 hours with 200IU IFN-γ is a well described phenomenon in the literature. We would like to refer to earlier publications of our group (Linnebacher et al., 2001; Schwitalle et al., 2004). Moreover, we include data on the upregulation of MHC I and HLA-A2 here, but not in the body of the manuscript, since this is, as said, a well known phenomenon (please have a look at those additional data). Additionally, MHC-I and especially HLA-A2-expression of LNCaP has been nicely analyzed by Carlsson and coworkers. We also include this manuscript. Finally, MHC-I and HLA-A2-expression data of Colo60H (a cell line with little data available in the literature) have also been included into the additional data presented to the reviewers.

5. Similarly to what the reviewer suggested for Tables 2 and 3, he asks for primary flow data on the intracellular expression of cytokines. Since these data are vital for the conclusions of the manuscript, we included those with the greatest explanatory power into the enhanced version of the manuscript (new Figure 4). Consequently, Table 4 has been removed.

6. The enhanced version of the manuscript has been reviewed by a native speaker. If this is not sufficient, the reviewer should please provide more information on the type of grammatical errors.

7. The reviewer asked for clear definitions of abbreviations used in the manuscript and especially in the figure legends. We carefully checked the use of abbreviations and modified the manuscript accordingly. We want to thank the reviewer for this very helpful comment, since there were indeed several mistakes. We regret this mistake.

Response to reviewer 2 comments:

1. We changed the term “growth factor” into “fold increase” as suggested by the reviewer. And we want to thank for this helpful comment.

2. The reviewer reminded us to avoid comments and interpretations of data in the results section. We agree with this second helpful comment and changed this as suggested.

3. Accordingly, the phrase “This formally proves that …” has been changed to “This proves that ….”

4. Similarly, “… contributable to …” has been changed to “This could mainly be attributed to the CD8+ T cells.” in the second page of the discussion.
5. The purification of B cells has been included into the Materials and Methods section “B and T cell purification. We regret this mistake.

6. Here, the reviewer asks for statistic evaluation of the data presented in Figure 4 (now Figure 5). We performed this analysis and included the results in Figure 5 and additionally included the description of the statistical procedure into the Material and Method section.

7. The inclusion of the statistical data on the reaction of the T cells towards FSPs also delivers the explanation, why we identified positive T cell reactions towards 4 peptides in this single analysis. Since this was one of four analyses performed in subsequent weeks, the total amount of significant reactions sums up to 6. We changed the discussion part accordingly.

We are very confident that the revised version now matches the requirements for publication in *BMC Cancer*. We would be very pleased if you and the first reviewer would find this enhanced version suitable for publication.

In the name of all authors,

Yours sincerely

Michael Linnebacher