Reviewer’s report

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

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Reviewer: Eyad Elkord

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This study investigated immune evasion mechanisms in a mouse model of colorectal cancer liver metastasis at different time points during tumour growth. This model was generated by intraportal injection of CT26.WT murine carcinoma cell line in Balb/c mice. The authors describe different immune escape mechanisms including immunomodulatory cytokines, expression of FAS/FAS-L and increase of T regulatory cells in a time-dependent manner during tumour growth. The findings in this study are as expected from other studies in animal models and colorectal cancer patients with liver metastases.

Major Compulsory Revisions

1- Materials and Methods are very detailed and should be shortened: For example culture of CT26.WT in pages 6 & 7; surgical procedure are well described in authors’ previous paper (reference 24). Also there are many unneeded details in other sections such as RNA extraction and real time PCR.

2- Results are generally not well described. Comparisons are not clear and p values should be indicated for every comparison as some differences are not significant such as CD25 at day 15 (figure 2a), TGF-b and TNF-a at day 15 (figure 3b), and FAS at day 15 (figure 4a). In figure 2a: Are authors comparing days 10, 15 and 20 to normal liver tissue? They should state that clearly in legend. Why Asterisk in figure 2b indicated on naïve? Why in figure 4b, no immunohistochemistry results for naïve are shown as for figures 2b & 3b?

3- It is not clear why authors show immunofluorescence data in figures 2c & 4d and all data presented in these two figures are from immunohistochemistry analyses. Alternatively, some immunohistochemistry photos as representative examples should be shown in figure 2.

4- Discussion is not very much related to the data shown in this study. The authors can discuss their results compared to other studies (e.g. reference 4). In page 16, the statement of “Thus, our results support the position that defective anti-tumor cytotoxic T cell responses contribute significantly to the suppression of an anti-tumor immunological surveillance” is not supported by the data presented here as no anti-tumour immune response was evaluated. In page 17, the statement of “We demonstrated that CD4+CD25+ T cells and CD8+ cytotoxic T cells were attracted by cytokines and chemokines as well as other factors as a part of the tumor microenvironment” is not demonstrated at all in this work.

5- There is a problem with references and authors have to check their relevance
carefully: For example in page 6 (reference 23), CT26.WT was not used in this study. In page 4, authors refer to one of their papers (reference 9) for inducible Treg, there are more relevant references. Similarly, reference 11 is not relevant.

Minor Essential Revisions
1- Was the model used xenomodel as described in the background of abstract?
2- In introduction, page 4, inducible Treg cells secreting IL-10 are Tr1 and not Th2.
3- Figure 1 is a very simplistic one and is not needed as data are described in details in page 13.
4- Day 20 on the photo of figure 2c should be made more clear.
5- Minor errors: In abstract, FOXp3 should be changed to Foxp3. Page 7: of all animals were (not was). Page 9: Albumin. Page 9: Fluoreszeinisothiocyanat looks German! Page 16: TGF-b and not anti-TGF-b.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.