Reviewer's report

**Title:** The efficacy of tumor debulking surgery is improved by adjuvant immunotherapy using imiquimod and anti-CD40

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**Reviewer:** Pablo Sarobe

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After reporting different combinations of surgery, chemotherapy and immunotherapy in previous papers, in the present manuscript authors analyze the anti-tumor efficacy of a combination of partial-debulking surgery with immunotherapy (Imiquimod+anti-CD40). Single and double adjuvant combinations are analyzed and immune responses and effector mechanisms are studied to explain the anti-tumor effects. The manuscript is interesting but several points need to be addressed:

**Major points:**

- Tumor experiments in Figure 2 show that combination of surgery with either Imiquimod or Imiquimod+anti-CD40 provide a higher survival benefit than surgery alone. However, are these treatments statistically different? One of the main concerns of these results is that despite a general trend of better results obtained when using surgery with immunotherapy (Imiquimod +anti-CD40), it is not clear whether combination with Imiquimod +anti-CD40 is better than combination with Imiquimod alone. Confirmation of these differences would clearly show that this combination is really needed.

- Keeping with the last comment, results in Figure 3 do not show an improvement when using surgery with Imiquimod+anti-CD40 as compared with surgery with Imiquimod.

- Finally, analyses of total and activated CD4 and CD8 T cells in lymphoid organs and tumors do not show the best results for surgery with Imiquimod+anti-CD40. Indeed, it is interesting that in previous comments surgery with Imiquimod was the second best treatment whereas in this case surgery with anti-CD40 seems to be better than surgery with Imiquimod. These results should be discussed in order to better understand the immune mechanisms responsible for the antitumor effect.

- Since combination including surgery with Imiquimod+antiCD40 is suggested as the best treatment, depletion experiments to characterize the role of CD8 T cells should be done in this setting, which would be more informative than experiments done using surgery with Imiquimod.

- Authors have previously reported (Broomfield et al Cancer Res 2005) that a combination of 75% debulking surgery with gemcitabine+antiCD40 has a survival efficacy of 70%, whereas in the present manuscript they reach a 30-40 %
survival. Given these results, which is the advantage of using the current strategy?

Minor points:
- Is Aldara cream directly applied i.t.? Also, authors indicate the advantage of i.t. administration due to surgery. However, in their model, Imiquimod is administered repeatedly. How feasible would this be in a clinical setting?
- Experiments in Supplementary Figure 1B-C do not add any information, since all these groups (except Imiquimod alone, whose effect has been already reported in previous publications) are included in Figure 2. Moreover, the surgery+Imiquimod group shown in Supplementary Fig 1 B-C and Supplementary 2 is exactly the same.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

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