Reviewer’s report

Title: PD-L1 assessment in pediatric rhabdomyosarcoma: a pilot study.

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Reviewer: Samuel Kerk

Reviewer’s report:

In this manuscript titled "PD-L1 assessment in pediatric rhabdomyosarcoma: a pilot study", Giulia et al characterized PD-L1 expression via immunohistochemistry and flow cytometry in both tumor cells and stromal cells in 16 primary patient RMS tumor samples, as well as in RMS cell lines. The authors report a lack of PD-L1 expression in RMS tumor cells, and positive expression of PD-L1 in the surrounding tumor stroma. Additionally, the authors report increased PD-L1 stromal expression in one patient previously treated with chemotherapy. The authors conclude that RMS patients may benefit from combination treatments with PD-L1 checkpoint blockade and chemotherapy. This manuscript could be improved by addressing the following points with further analyses or discussion.

1. The greatest limitation in this study is the small sample size. As the authors themselves mention, no statistical tests of significance could be conducted due to the small cohort size. Could the authors access additional RMS sample to examine PD-L1 expression? This would increase the statistical power of this study.

2. H460 cells were used as positive controls for the flow cytometry analysis of PD-L1 expression. Do the authors also have both positive and negative controls for the IHC analysis in Figure 1 of PD-L1 expression?

3. In Figure 2, the authors show that PD-L1 expression co-localizes with the tumor immune cell compartment. Could the authors also show a lack of co-localization between PD-L1 and tumor cell markers?

4. Do the authors have access to more patient biopsies before and after treatment with chemotherapy? Figure 3 shows an increase in PD-L1 expression following chemotherapy, however this is just one sample. Therefore, it is difficult to conclude that chemotherapy could ignite inflamed tumors.

5. Did the authors investigate any possible correlation between PD-L1 expression in the immune cell compartment and patient outcomes?

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes
Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

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