Reviewer’s report

Title: Smac mimetics LCL161 and GDC-0152 inhibit osteosarcoma growth and metastasis in mice

Version: 0 Date: 17 Jun 2019

Reviewer: Christian D Young

Reviewer’s report:

In the manuscript "Smac mimetics LCL161 and GDC-0152 inhibit osteosarcoma growth and metastasis in mice and enhance the efficacy of doxorubicin" composed by TM Shekhar and colleagues, the authors explore the use of Smac mimetics to treat mouse models of osteosarcoma. They follow up on a previous publication where they demonstrate, in vitro, that smac mimetics reduce osteosarcoma cell survival in a TNFalpha dependent manner. In this manuscript under review at BMC Cancer, the authors perform well-described, well-controlled experiments in nude mouse osteosarcoma transplant models, with supporting experiments in cell culture and a genetically-driven spontaneous mouse model of osteosarcoma. Most of the authors conclusions are supported by data, and after addressing a couple major concerns (that I feel will be easily addressed) and a few minor concerns, this manuscript will advance our knowledge of possible osteosarcoma treatments and the use of smac mimetics.

Major concerns

1) The authors use two way ANOVA in several instances, and this is not correct. Most of their data should be analyzed by one way ANOVA since they are not testing two independent variables.
   a. Figure 1: All data should be analyzed by one way ANOVA with multiple comparison testing. Especially, since all data is compared to saline, there is only one variable.
   b. Although some may consider a four arm experiment of no drug, drug 1, drug 2 vs drug 1 & 2 as two variables, it is really one variable: treatment. This should also be tested with one way ANOVA (either repeated measures or testing time points of interest on their own).
   c. Check all instances of two way ANOVA usage: I don't think any are appropriate

2) The authors’ conclusions for results presented in figure 2 are probably not substantiated by the data. To be clear, I think transparent presentation and conclusion drawn from the data is the important component here and it will not change the overall conclusion of the manuscript, but it may for this figure. Again, the two way ANOVA is not appropriate. And, more importantly, the authors claim that the smac mimetics "cooperate with
doxorubicin", yet the statistics are compared to saline. Figure 1 basically demonstrates that the smac mimetics have anti-tumor effect, but the doxorubicin effect is minimal. It would follow that they would be testing whether doxorubicin + smac mimetic have better anti-tumor activity than smac mimetic alone. Thus, treatments should not be compared to saline. I suspect that the double treatment may not be better than smac mimetic alone, in the 1029H model, and this is what should be stated and discussed. This lends credence to new drugs, like smac mimetics, over standard of care.

Minor concerns

3) Readers would benefit from an explanation of what they are looking at in the PET and MRI images in figure 1C. Is this a whole mouse with part of the image representing the tumor? Is this a whole tumor? What do the colors or greyscale represent? Arrows pointing to described areas, a key and/or heat map details or cartoon would be helpful.

4) As discussed above, present statistics on smac mimetic alone vs smac mimetic + doxorubicin in figure 2.

5) The described gating in figure 3 legend does not necessarily make sense for the described cell types. F4/80 as the sole marker of macrophages is probably ok may be a stretch. Neutrophils should be positive for Ly6G. Either describe more clearly, or describe the cells by their markers. I think the overall conclusion that myeloid cells are present and contain TNFalpha will be maintained. In discussion, the authors are careful to not dwell on a particular TNFalpha-expressing cell and it may be best to present the data that way if they can re-gate to show myeloid cells as expressing TNFalpha. Or, double check that their gating is truly representing macrophages, NK cells and neutrophils.

6) The authors discuss statistical results in Figure 4a legend, but no statistics (*) are presented in the figure panel

7) Clarify how a luminescence-based viability/proliferation assay is affected by luciferase positive cells

8) It is unclear what statistical comparison is being made in figure 5c. Is it pairwise comparison of human vs mouse TNFalpha? If so, one way ANOVA cannot be used. Mann-Whitney or T test would be appropriate

9) Figure 6c is unclear as presented. I think it would be easier to interpret if the data were separated into two panels: C) KRIB-Luc cells per lung in each treatment group; D) Bioluminescence in each treatment group. Again, if doxorubicin + LCL is not better than LCL alone (or dox alone in this case), then discuss this. This would not alter the overall conclusion about smac mimetics, but may alter the conclusion about combination and risk of combination. Comparing to saline in Fig 6a is not terribly helpful.

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.

Yes

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

Yes

**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

**Quality of written English**
Please indicate the quality of language in the manuscript:

Acceptable

**Declaration of competing interests**
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?
If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal