Reviewer’s report

Title: A new anti-glioma therapy, AG119: Pre-clinical assessment in a mouse GL261 glioma model

Version: 3 Date: 20 April 2015

Reviewer: Lara Collier

Reviewer’s report:

1. Is the question posed by the authors well defined?
   Yes and it is an interesting question.

2. Are the methods appropriate and well described?
   Mostly. There are some discrepancies that should be corrected and some details that it would be beneficial to add:
   A) The methods say 5-6 mice per group where figure legends indicate an n of 7 for AG119.
   B) It would be good to document how antibody therapies are delivered (IP?).
   C) Line 158 states that “euthanized 1-2 days prior to expected disease-initiated deaths”. It would be good to expand upon the criteria that were used to determine that the mice were moribund since survival analysis is used as one measure of therapy efficacy.
   D) For viability assays, from the methods it is unclear if concentrations of vehicle (DMSO) were controlled for in experiments to determine the IC50 of TMZ and AG119. Although the amounts of DMSO added to cells for AG119 studies are calculated to be minimal; for TMZ studies it seems like high potentially detrimental concentrations of DMSO would be present in cells treated with higher amounts of drug. If not controlled for, this could lead to an under-estimation of IC50 values.
   E) For animal studies were non-treat animals injected with vehicle or saline as a control? If not could this influence results?

3. Are the data sound?
   Yes.

4. Do the figures appear to be genuine, i.e. without evidence of manipulation?
   Yes.

5. Does the manuscript adhere to the relevant standards for reporting and data deposition?
   Yes.

6. Are the discussion and conclusions well balanced and adequately supported
by the data?
Mostly. However in a couple of instances it appears that the conclusions may somewhat overstate the data.

A) In line 205 it is stated that “AG119 compared well against other anti-glioma therapies including anti-VEGF and anti-c-Met antibody therapies, or TMZ, regarding both survival and tumor volumes.” Given that AG119 was inferior to TMZ in survival, this statement should be updated to reflect that fact.

B) In line 217 it would be clearer to phrase it that T98G and U251 cells had similar sensitivities to AG119 (As opposed to saying “not resistant”).

C) In line 241 it is stated that “In this work we showed that AG119 is also not subject to MGMT mediated resistance, as is the case with TMZ.” This is based on the fact that both T98Gs and U251s have a similar IC50 value for AG119. However to nail this down definitively, the authors would need to show that T98Gs and T98Gs with MGMT knock-down are equally sensitive to AG119 (and a similar experiment for TMZ over-expression in U251s). This reviewer does not feel that these experiments are necessarily required for publication of this paper, but feels that the discussion should be modified to indicate the limitations of the experiment.

7. Are limitations of the work clearly stated?
See above.

8. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
Yes- although in reference 13, AG119 appears to be referenced to as 3-HCl. It would be good to add that detail to the introduction.

9. Do the title and abstract accurately convey what has been found?
Yes, except see discussion about MGMT/TMZ above.

10. Is the writing acceptable?
Mostly. There are discrepancies that should be corrected and some suggested changes that would make it easier for a reader to follow the paper.

A) In line 58 it might be clearer instead of “have not improved overall survival” to say “have not substantially improved overall survival” to indicate the fact that studies have found that temozolomide added to radiotherapy extended survival by 2 months compared to radiotherapy alone. A similar change is suggested in line 114 as well.

B) In line 68 should the p value <.05 really be <.001 as indicated in Figure 1?
C) In line 70 should the “and” before anti-c-Met be deleted?

D) Figure legend 1 should be updated to include the anti-c-Met and anti-VEGF groups that are shown on the graph.
E) The Results/Discussion begins rather abruptly. It would also help the flow of the article if the various results/discussion were split into new paragraphs with a
sub-heading and a sentence or two added to briefly summarize the model employed and the experimental design.

F) Overall, the writing about perfusion rates is hard to follow. It seems in certain instances that the word “decreased” is used when it is actually referring to the ability of tested drugs to increase perfusion rates in tumors compared to no treatment.

**Level of interest:** An article of importance in its field

**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