Reviewer's report

Title: Targeting and killing of glioblastoma with activated T cells armed with bispecific antibodies

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Reviewer: Tiffany Doucette

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Manuscript Overview:
In the study by Zitron et al. entitled “Targeting and killing of glioblastoma with activated T cells armed with bispecific antibodies”, the investigators aimed to show that activated T cells can be used as a potential immunotherapeutic for targeting and killing glioblastoma cells. The authors show that armed activated T cells (ATC) with bispecific antibodies targeting HER2 or EGFR decreased cell viability and increased cytotoxicity in several glioma cell lines and in primary glioblastoma cell lines. Additionally, the authors look at the ability of the armed ATC to kill glioma cells when given simultaneously and whether armed ATC could still be effective against chemo-or radio-resistant cells. While this study does lend support for the potential of armed ATC cells as a new treatment option, several inconsistencies exist and need to be further examined and explained.

Discretionary Revisions:
1. The Introduction of the manuscript could provide a little more background and data from previous studies using armed ATC with bispecific antibodies to show effectiveness of their use in other cancer models.
2. In Materials and Methods, culturing of both primary and long-term cell lines was done in “DME-F12-based medium”. Is this the same as DMEM-F12 and if so, should be changed since it is commonly written as such.
3. For consistency in the figures, show statistical significance if it is performed.

Minor Essential Revisions:
1. On Title Page, it appears that the first name of author Lum is incomplete.
2. A description of the results for HER2Bi should be included under “Optimizing the Arming Dose Bispecific Antibody” in the Results section.
3. Any time a statement is made about results that were obtained, there should be a reference to a figure showing the results or label it as “data not shown”. This occurs multiple times in the manuscript.
4. Figure 2: Please re-label the x-axis so that the E:T ratio is clearer (i.e. 1.5:1, 3:1, etc.).
5. Please provide a reference describing proliferation of cells in the presence of ATC.
6. Under Results in the section about the CD133 cells, the word “be” should be
removed from the section title.

7. Under Results, “Does chemoresistance confer……?”, 4th line in the paragraph, remove the word “that” and provide a reference for the other studies showing radioresistance of armed ATC effector function.

8. Under Results, “Does chemoresistance confer……?”, 3rd sentence in the second paragraph should read “Radiation alone had no effect on the viability of either …….”.

9. Under Results, “Do glioma cells exhibit suppressive or contact-dependent inhibitory effects on ATC?”, the 3rd sentence, “did not inhibit the ability of ......” to do what? Also should be included in the figure. Why was U188MG used only for that small test?

Major Compulsory Revisions:

1. If Supplementary Figure S1 showing MTT responses of the glioma cell lines, then it needs to be clearer. In the Results section, there is more description about other cell lines and the results when subjected to the armed ATC. It is stated that the authors have employed the 51Cr release assay in previous work with armed ATC but there is no reference or indication to show comparison of the results. If both assays are utilized in the study, then proof of validation for both should be included or at least referenced to previous work.

2. Figure 1 (top panel) is incomplete. There should be an unarmed control and the results of the other two target cell lines should be shown at least in supplementary data. How was the E:T ratio of 5:1 determined to be used? The data for the irradiated armed ATC should be shown as well since it is stated that there was “minimal effect on their activity”. Part of your goal was to show that irradiation doesn’t affect the ability of the armed ATC to target and kill glioma cells. One solution could be to make separate graphs for the MTT assay and the 51 Cr assay and plot all cell lines (irradiated and radiated) on each. Also, why does the viability increase in cells treated with HER2Bi at the higher arming dose?

3. Figure 1 (lower panel): Authors do not comment on why there is a slight reduction in the U87MG cell line when treated with CD20Bi but actually state that it “showed no reduction of tumor cell viability”. Another statement was made to results from separate experiments with no reference or data not shown implicating where the results came from. Not sure why the last sentence in the Specific Killing of Long-Term Glioma…… Results is included. If it is believed that examining the effects of soluble antibodies on the glioma cells is important, then this should be included in the methods, a figure and the discussion.

4. Figure 2: There are discrepancies in the figure legend and the results on how many experiments the data was pooled from and it is unclear about the number and use of the ATC donors. Please clarify. The figure depicts results from one ex vivo cell line showing a slight difference between killing by HER2Bi and EGFRBi. Is it known whether expression of HER2B was higher in these cells to account for this?

5. Data on simultaneous use of BiAbs: The fact that primary glioma cells from
only two patients were used in this experiment and this experiment wasn’t done in the three long-term glioma cell lines does not provide enough support in the conclusion that these BiAbs do have an additive effect. As the authors mentioned before, there may be varying levels of expression of HER2 and EGFR among different patients and in the long-term cell lines. The importance of the significant difference in the EGFRBi-armed cells compared to the double- or mixed single-armed ATC should not be downplayed with the limited sample size. It is important to know what the expression levels were for HER2 and EGFR in both of these primary glioma lines to see if one was substantially higher than the other.

6. Figure 4: Explain why the E:T ratio of (3:1) was used instead of 5:1 used in the previous experiments. Also, where is the data on the effect of EGFRBi armed ATC on these cells?

7. Figure 5: Again, explain the use of a different E:T ratio (this time 10:1). How many times was this experiment repeated? A cell line not resistant to TMZ should have been used as a control. Were the results similar in the other cell types including primary glioma cell lines? For consistency, please show significance symbols on the graph. It needs to be clearer about whether you are comparing treatments to unarmed effects or treatments to each group’s Rad-TMZ- column when you are reporting your results in the results section. The authors also suggest that TMZ may be potentially toxic to ATC. Was a viability assay done on unarmed ATC with or without treatment of TMZ to show this?

8. Supplementary Figure S2: Why was only HER2Bi tested and data shown for only one cell line? Authors state that co-culture with glioma cells did not suppress the ability of armed ATC to kill glioma cells but do not show the data or reference “data not shown”. No statistics are shown for this figure. Additionally, it appears that there is some reduction in killing of HER2Bi/U251 cells after first contact with SKBR3 even though the authors state that there was no reduction.

9. Figure 6: Again no explanation on difference in E:T ratio used. The authors only show data for EGFRBi-armed ATC and not HER2Bi-armed ATC. Only one cell line used and no explanation for why. No statistics shown for the results.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests.