Reviewer's report

Title: The bacterial protein toxin, cytotoxic necrotizing factor 1 (CNF1) provides long-term survival in a murine glioma model

Version: 1 Date: 17 April 2014

Reviewer: Paolo Malatesta

Reviewer's report:

The study from Vannini and colleagues describes the effects of a bacterial toxin (CNF1) on glioma cells and reports its efficacy in prolonging survival of mice orthotopically transplanted with a murine glioma cell line (Gl261). The results are of potential great interest but some issues need to be addressed before the paper is suitable for publication.

Major Compulsory Revisions:

Two issues to be clarified involve the paragraph about the fate of Gl261 after prolonged CNF1 exposure:

1) First is not clear why the AnnexinV and PI analysis were made 10 days after treatment. Authors stated that this time point is "during the period of GL261 cell loss", but from the histogram in fig4A, it seems there is no cell loss up to day 12, while a dramatic loss occurs between day 12 and day 15. Letting apart the fact that such sudden and synchronized cell loss is very singular and may deserve a deeper analysis, this behaviour is compatible with a massive apoptosis initiated AFTER day 10. The analysis should therefore be repeated at a more relevant time-point, in alternative the choice has to be carefully and convincingly justified.

2) Moreover, it is obscure how "the majority of CNF1-treated cells at 10 days resulted positive for both annexinV and propidium iodide" (page 10, third-last row), while, in the meantime, less than 20% of CNF1-treated cells were PI-positive and only about 25% were annexinV-positive.

Probably the authors are referring to some kind of subsetting (may be they mean the AnnexinV-positive cells among the PI-positive cell, as suggested by the labelling "AnnV/PI" in the figure?) but this is not clearly explained neither in the text nor in the legend. If my interpretation is correct, however, it is still not completely obvious the possible meaning of the observation.

3) Some technical problems seem to affect the Kaplan-Meier curve for the controls animals (that is the same in Fig7B and Fig8A -and this fact should be clearly indicated-). First, presuming that the size of a single event is that visible at about 16 days, there is a strange "ministep" at about 32 days, that is smaller than a single event and make no sense. In addition, the sum of all the depicted steps (see attached graphic scheme) gives a total of 19 events instead of 16 events as it is stated in the text (page 11, second last row), either the graph or the text should be corrected.
Minor Essential Revisions:
4) In my understanding, histogram in fig2C and fig2D are redundant because they show the same data: cells are either mono or multinucleated cells. The percentage of multinucleated cells is always 100% minus the percentage of mononucleated cells. The authors should decide which side they want to show.

5) In the introduction a four groups classification for gliomas is mentioned but is unclear to which classification scheme the authors refers to. The statement should be either elaborated or omitted.

6) The median survival of glioblastoma patients indicated in the introduction should be updated.

Discretionary Revisions:
7) The whole message of the paper would be dramatically improved if the in vivo analysis would not rely uniquely on a cell line that is known to be immunogenic (although weakly) and that (likely for this reason) it has been efficiently eradicated by different treatments in other occasions (see for example Daga et al. Int.J of Cancer 2007, Kjaergaard et al. Cancer Res 2000). It would be strongly advisable to test CNF1 either on an additional glioma model, or, even better, on the very same Gl261 transplanted in an immunodeficient mouse strain to have a more realistic assay of the efficacy of the toxin.

8) The pictures of the wound healing assay are not particularly good in quality. Gray-scaled pictures would be more appropriated.

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