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:** 16 April 2014

**Reviewer:** Benjamin Pineda

**Reviewer's report:**

**Major Compulsory Revisions**

This paper describe the potential use of bacterial protein toxin, cytotoxic necrotizing factor 1 (CNF1) as a potential antineoplastic treatment.

Figure 6 "untreated cells" is out of focus and do not allow to differentiate if CNF1 induces multinucleation in human primary tumoral cells. Also, some cells look like they were bi-nucleated, therefore, authors should improve this figure.

Regarding the toxicity of CNF1, authors argue that CNF1 produces a long-lasting activation of Rho GTPases and catalyzes the deamidation of a single glutamine residue of the Rho GTPases (RhoA, Rac1 and Cdc42) as a main cytotoxic effect. However since CNF1 comes from E. coli, it is important to consider the possible induction of autophagy by ligation of TL4 [due to it mediates many biological effects of lipopolysaccharide (LPS)] and this ligand is expressed in some human glioma tumors and cell lines of glioblastoma. This is the case of the GL261 cell line. I consider authors should argue this possibility.

On the other hand, in the in vivo experiments authors do not consider the possible participation of inflammation induced by innate immune response such as cause to reduce tumoral volume and increase survival in mice treated with CNF1.

Also, it is important consider that the contamination by LPS or other bacterial products could be a trigger to inflammatory response and autophagy induction by TL4, it is important monitoring the amount of LPS and endotoxins present in their toxin, due to the toxin is derived from E. coli.

Some references that could be help are:

Concomitant treatment with pertussis toxin plus temozolomide increases the survival of rats bearing intracerebral RG2 glioma.

Magaña-Maldonado R, Manoutcharian K, Hernández-Pedro NY, Rangel-López E, Pérez-De la Cruz V, Rodríguez-Balderas C, Sotelo J, Pineda B.


Role of autophagy in temozolomide-induced cytotoxicity for malignant glioma cells.

Kanzawa T, Germano IM, Komata T, Ito H, Kondo Y, Kondo S.
Intratumoral injection of lipopolysaccharide causes regression of subcutaneously implanted mouseglioblastoma multiforme.


**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