Reviewer's report

**Title:** Adeno-associated Virus-mediated Doxycycline-regulatable TRAIL Expression Suppresses Growth of Human Breast Carcinoma in Nude Mice

**Version:** 2  **Date:** 8 February 2012

**Reviewer:** Liang Cao

**Reviewer's report:**

The authors developed a rAAV-based viral vector system with TRAIL expressed under a Tet-On system. This work is a continuation study of previous publication in which rAAV-TRAIL non-inducible vector has anti-tumor activity. The methods described in the manuscript are appropriate to the questions the authors tried to answer and results appear to be reasonable. However, there are some significant problems that must be addressed by the authors before the manuscript should become acceptable by BNC cancer for publication:

1. If this work is to serve as the preclinical work for the development of this inducible vector, then the results fail well short of what is required. The viral vector is minimally active in vitro (Fig 2), and performs poorly in vivo (Fig 4). It is much worse than the anti-tumor activities of Apo-2L (Genentech’s TRAIL ligand) or Drozitumab (Genentech’s DR5 antibody), in many cases, against the same cancer cell lines. Both agents are in clinical development. These clinical trial agents resulted in tumor size reduction with durable response in various animal models. Nothing indicates that this is the case for Tet-On rAAV-TRAIL. At the very minimal, the authors should compare the TRAIL produced by their vectors with a recombinant human TRAIL at a comparable concentration to show that rAAV vector TRAIL has comparable avidity against a panel of tumor cell lines. Preferably, a non-inferior animal study should be performed. There is little development value if the rAAV vector TRAIL is not sufficiently or equally active.

2. The main problem with current clinical development of death receptor targeted agents is the lack of efficacy. It is not clear that Tet-On rAAV-TRAIL would be any better. Where is value proposition?

3. Results clarification
   a. Results of Fig 2A (24 hrs) and Fig 2B (time course) are inconsistent, in which in many cell lines only show difference after 72 hrs.
   b. MTT assay is not sufficient for the in vitro studies. Other results will be required, including the demonstration of percentage of cells with nuclear fragmentation, and complete cleavage of caspase-8,-3.

4. The quality of the figures need to be improved substantially:
   a. Condense each figure to one page, e.g., panels of A, B, C, and D of Fig 1 need to be on a single page.
b. Number each figure correctly.
c. Improve the quality of cell images of Fig 1B and Fig 3B.
d. Fig 2A, X-axle number 1, 2, 3, 4 should be replaced by word legends and X-axle title should be added to each panel.
e. Annotate NTC & PTC in Fig 3A and Fig 4B.
f. Line up b-actin to the corresponding samples in Fig 3A

5. Overall English, include grammar and spelling need to be proof-read by a native-speaker.

6. Several specific errors, e.g., the last sentence of 1st paragraph on page 10 stated that ‘These data indicate that Tet-On system can strictly control target gene expression driven by either TRE or CAG promoter’, the results of Fig 1 discussed in the paragraph did not support the controlling of CAG promoter. Mismatch of figures discussed in the Results section with figure legends. Redundancy of word, ‘gene’ in line 4 on page 7. In the 3rd paragraph of discussion section on page 14, the authors stated ‘…showed that sTRAIL protein was predominantly expressed in liver and tumor tissue under induction condition’, but there is no results presented in the manuscript related to this conclusion.

**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:**

None