Reviewer’s report

Title: Expression of RNA interference triggers from an oncolytic herpes simplex virus results in specific silencing in tumour cells in vitro and tumours in vivo.

Version: 2  Date: 28 April 2010

Reviewer: Oliver Ebert

Reviewer’s report:

The authors’ revision of the original manuscript clarified some of the issues initially raised. However, several points have not been sufficiently addressed (Major Compulsory Revisions):

1. The authors argue that since the direct oncolytic effect of HSV is impaired by its attenuated ability to infect and replicate in some tumors, the therapy can be improved by incorporating an RNAi targeted to a secreted tumor protein. However, the copy number of the RNAi trigger would also be inhibited in these cells due to low/moderate levels of infectivity/replication. Therefore, the question of whether or not this strategy would be effective in these tumors remains to be answered. The only way to sufficiently answer this question would be to incorporate one of the proposed therapeutic RNAi triggers into the virus.

2. It is unclear why the authors use quantification of b-gal concentration as a measure of cell survival. MTT assays are well established and standardized and would provide much more informative data than relying on the amount of b-gal expression of the stable cells lines to reflect cell viability. It has been demonstrated that HSV infection results in a shut-down of translation of host cell proteins, and even if this shut-down is only partial, I would expect a shift in translation levels to favor anti-viral responses. Therefore, a drop in a b-gal expression in virus-infected cells (increasing over time and in direct proportion to MOI) does not necessarily indicate cell death, but perhaps the cellular response to infection.

3. The argument that “there is no reason to suspect” that insertion of short RNAi sequences would influence the growth or cytotoxicity of the virus, is not really scientifically acceptable. When presenting scientific data it is always important to include the necessary controls, even when the results are predicted. To include an additional panel representing the control vector would involve a minimal amount of work and answer some questions. Just because the kinetics of the shRNA- and miRNA-encoding vectors result in similar kinetics does not mean that they are similar to that of the backbone.

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.