Reviewer's report

Title: Human papillomavirus (HPV) type 16 E7 protein bodies cause tumour regression in mice

Version: 1 Date: 27 September 2013

Reviewer: John B. Liao

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Major Compulsory revisions

1. The reason that insect cells and baclovirus are used to isolate Zera protein bodies, if one of the proposed advantages of using Zera is to express recombinant protein in whole plants, should be clarified.

2. eGFP has been reported to be immunogenic in mice. The rationale for including this as a control in these experiments should be clarified. (Figure 3-4)

3. The reason tumors were run for 5-6 weeks in Figure 5, but only 2 weeks in Figure 3 should be explained further. The way these experiments are reported raises the question whether differences not seen in some of the groups in Figure 3 would have become evident if tumors were propagated longer. The presentation of a single timepoint and 2 identical experiments (Figure 5) is less informative than the plots over time seen in Figure 3.

Minor Essential Revisions

1. Figures (3-7) and/or figure legends should show p values and clarify which timepoints or comparisons are statistically significant.

2. Nomenclature of vectors should be made consistent between text and figures.

Discretionary revisions

1. This manuscript could be improved if it were more focused on the role of Zera as a novel vaccine adjuvant rather than use as potential therapeutic cervical cancer vaccine, which is what the introduction currently prepares the reader for.

2. Although immunogenicity and tumor control are seen when mice are vaccinated with a shuffled HPV 16 E7 sequence, the presence of E749-57 in the vaccine as a preferred H2Db restricted epitope is likely responsible most of tumor control and ELISPOT and Granzyme B results. How inclusion of this epitope in the vaccine this limits translation of these findings clinically to humans with outbred MHC class I alleles may be discussed.

3. A rationale proposed for these experiments centered on the use of Zera sequences and plant expression to allow high-level and inexpensive production
of a therapeutic protein based vaccine targeting HPV 16 E7. However, data presented from these experiments suggest that DNA based vaccination produces superior tumor control (Figure 5) and comparable immunogenicity (Figure 6). The discussion could explore the ramifications of this in more depth.

**Level of interest:** An article whose findings are important to those with closely related research interests

**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.