Reviewer's report

**Title:** The use of biofluorescence and high-frequency ultrasound imaging for in vivo evaluation of gene therapy vectors

**Version:** 1  **Date:** 22 July 2013

**Reviewer:** Dominic Wells

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This study reports the use of high-frequency ultrasound (HF-US) combined with biofluorescence imaging (BFI) compared to traditional in vivo calliper measurements to determine the impact of a Herpesvirus saimiri amplicon on human colorectal cancer xenograft growth.

The results are interesting and relevant to many tumour studies. In general the experiment is well described and analysed but there are some areas of concerns and/or lack of information.

**Major Compulsory Revisions**

1. The authors report a number of mice lost from the control group (Figure 1) but do not give the number. In addition, unless some of the data points are overlying there appears to be a variable n values in the graphs shown in Figures 2 and 3. The authors should state the exact n values in each figure legend.

2. The Bland-Altman figure legend needs more details to explain how the average volume and the bias were calculated. Why are the n values different for the HF-US and in vivo calliper measurements?

3. The correlations for Figure 7 are highly skewed by the single high value data point and as such are highly questionable. I recommend deletion of this figure and associated text as I do not consider the correlations to be reliable given the skewed data.

4. What sex of mice were used?

5. There is no discussion of the pros and cons in terms of animal welfare. The calliper method requires restraint whereas the images methods require general anaesthesia. As the authors noted the principle of the 3Rs in the introduction I feel this point should be discussed.

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

**Quality of written English:** Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests.