Reviewer’s report

Title: Assessment of anti-inflammatory tumor treatment efficacy by longitudinal monitoring employing sonographic micro morphology in a preclinical mouse model.

Version: 2 Date: 10 March 2011

Reviewer: James Lacefield

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Major Compulsory Revisions

1. [Results, 2nd paragraph and Fig. 1A]: It is not clear whether the observed difference in growth rate of the two groups from Day 7 to 12 is a meaningful result because the tumor volumes on both days are not significantly different, so the apparent difference in slope may simply be the result of volume measurement variability. The fact that the mean control tumor volume was less than the mean treated tumor volume on Day 7 in particular gives the impression that measurement variability was a factor here. Is a difference in slope still observed if the change in volume of each individual tumor is computed and then those volume changes are averaged, as opposed to taking the difference of averaged volumes?

2. [Results, 2nd paragraph and Fig. 1B]: What is the likely explanation for the fact that the variability of the control tumor volume was greater than the variability of the treated tumor volume, but the variability of the treated tumor mass was greater than the variability of the control tumor mass? This apparent inconsistency was not observed in the second (resected model) study (Fig. 2B).

3. [Discussion, 1st paragraph, last sentence]: What is the basis for declaring longitudinal ultrasound measurements to be "more reliable" for monitoring therapeutic efficacy? Presumably the comparison here is to the end-point tumor mass. The statistical significance of the difference in end-point mean tumor volume and end-point mean tumor mass was the same in the second study, and as pointed out above the variability of the volume measurements was not necessarily better than the variability of the mass measurements.

4. [Discussion, 2nd paragraph]: It is not clear that these studies actually demonstrate detection of "complex" tumor growth kinetics. Figs. 1A, 2A, and 3 appear to show exponential or even linear tumor growth; it is debatable whether any of the longitudinal data sets qualifies as complex.

Minor Essential Revisions

1. [Methods, 4th paragraph, minor issue not for publication]: The axial spatial resolution of the 40- and 25-MHz transducers is 40 and 70 microns (not mm), respectively.
2. [Methods, 5th paragraph]: Please specify the spacing between image planes that were segmented to estimate tumor volume as this is a user-selected parameter in the VisualSonics software that can affect the accuracy and variable of the volume estimates.

3. [Results, 1st paragraph and elsewhere, minor issue not for publication]: The precision to which most of the p values are reported suggests that these are the actual p values, not the threshold of statistical significance, so these data should be reported as, e.g., p = 0.016, not p < 0.016.

Discretionary Revisions
1. [Results, 5th paragraph]: Consider including a scatter plot with the best-fit regression line to illustrate the comparison of ultrasound tumor volume and end-point tumor mass. Why was a volume-to-mass correlation coefficient not reported for the first study?

2. [Results, 6th paragraph]: Sample images showing the ultrasonic appearance of a representative tumor and surrounding pancreatic tissue would increase the usefulness of the paper to other researchers who are considering using ultrasound to study mouse pancreatic tumor models by helping those readers understand what to look for in their images. Sample images of the peritoneal tumors might be the most interesting as this seems to be the most significant advantage of ultrasound over conventional end-point analysis in this study.

**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:**

The reviewer has received in-kind research support from VisualSonics Inc., the manufacturer of the ultrasound system used in this study.