Reviewer's report

Title: Effects of ATRA Combined with Citrus and Ginger-Derived Compounds in Human SCC Xenografts

Version: 2 Date: 12 April 2010

Reviewer: Wenyin Shi

Reviewer's report:

- Major Compulsory Revisions

The author must respond to these before a decision on publication can be reached. For example, additional necessary experiments or controls, statistical mistakes, errors in interpretation.

1. The authors used very small sample size for their in vivo studies. 7-8 animals per group is acceptable, 5 animals per group for xenograft study is too low. More importantly, when dealing limited number per treatment group, one can no longer assume the distribution of data is normal. Therefore, mean+/-SE can no longer be used to represent the group. The appropriate way is to use median and perform non-parametric statistical analysis. All the in vivo experiment data need to be re-analyzed. The results can be significantly different from presented.

2. One of the authors' arguments for the combination study is to limit toxicities associated with ATRA (first paragraph of discussion). However, according the animal weight data, all the combination groups of ATRA and ACA were associated with significant weight loss, ATRA 10 and AUR+ATRA groups also had some significant weight loss. These data, at least at the doses used in the paper doesn’t support the claim of limit ATRA toxicity by combination with ACA or AUR.

3. It is unclear why animals need to be pre-treated for 1 week. Such treatment may potential affect the tumor take rate, viable tumor stem cells, and animal health status. Such design makes the assessment of the direct effect on tumor growth more difficult. The more appropriate design would be injecting animals with tumor cells. Then randomize the treatment group when tumors reached a predefined size (such as 0.1 cc). Then start treatment. This is will direct assess the diet effect on tumor growth, without other confounding factors. The author needs at least discuss the rationale of such experimental design, since the purpose of the current study is to evaluate the antitumor effect rather than tumor prevention effect.

4. Three different doses of ATRA were used. Were there significant differences between these 3 different doses? Please provide data.

- Minor Essential Revisions

The author can be trusted to make these. For example, missing labels on figures,
the wrong use of a term, spelling mistakes.
None.

- Discretionary Revisions
These are recommendations for improvement which the author can choose to ignore. For example clarifications, data that would be useful but not essential.
1. The data were truncated at day 25. Were the animals all killed at that time, for the animals in the treatment groups with significantly smaller tumors, were they continue to be followed?

**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