Reviewer’s report

Title: Cucurbitacin B inhibits human breast cancer cell proliferation through disruption of microtubule polymerization and nucleophosmin/B23 translocation

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Reviewer: Gail Fraizer

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This clearly written paper has been improved by providing additional data and clarifying some confusing statements.

Specific comments addressed:

1. The immunofluorescent images in Figure 6, have been improved and now show clearly the controls have Nucleophosmin/B23 in the nucleolus and it is shifted to nucleoplasmin in treated cells. In contrast to the location changes, the quantity of NPM appears less affected by treatment.

2. The legend for Figure 5 is improved and, importantly, quantitation of the western (5B) is now included in the supplemental data. However this supplemental data needs to be cited in Figure legend or in results (currently it is not). Alternatively the quantitation could be added as panels 5C and D.

3. The authors have restated their belief that the lack of evidence of disruption of microtubule polymerization in vitro shown in Figure 8B, does not preclude disruption in vivo. Although alpha tubulin staining is not clearly visible in control MCF-7 cells, it is clearly visible in treated MCF-7 cells; and in MDA-MB-231 cells the pattern does appear to change from diffuse cytoplasmic (in untreated controls) to aggregated cytoplasmic staining in treated cells. Thus, although staining pattern is still difficult to interpret, the authors have demonstrated altered microtubular staining in treated MDA-MB-231 cells.

Minor Typos:

In discussion 2nd paragraph Pg 13 refers to staining showing disruption of microtubules as Figure 6a, whereas Figure 8a is correct citation.

Conversely 1st paragraph Pg 14 refers to translocation of NPM as Figure 8, whereas Figure 6 is correct.

Conclusion:

Overall the manuscript provides compelling evidence for the utility of Cucurbitacin B as an adjuvant therapy. Results presented show very clear evidence of the anti proliferative and apoptotic effect of Cucurbitacin B on breast cancer cells. Most data is consistent and confirmed by multiple approaches in two breast cancer cell lines. Mechanistic questions are addressed by experiments that show 1) the reduction of NPM and myc protein levels by Cucurbitacin B, 2) the translocation of NPM following Cucurbitacin B treatment,
and 3) in one cell line, the visible rearrangement of alpha tubulin induced by Cucurbitacin B treatment.

**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