Reviewer's report

Title: Pharmacological targeting of valosin containing protein (VCP) selectively kills canine lymphoma cells by inducing DNA damage

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Reviewer: Yihong Ye

The paper by Nadeau et al studies the anti-cancer activity of the p97 inhibitor Eeryarestatin I (Eey1). The authors examined the expression of p97 in canine B-cell lymphoma cells and found that p97 expression correlates with tumor malignancy. They then tested whether canine lymphoma cells are more sensitive to Eey1-induced cytotoxicity, which turns out to be the case. This observation is consistent with several published reports, showing that Eeryarestatin I has broad anti-cancer activities (Wang, Q. 2000 PloS One; Chou TF 2011 PNAS).

Surprisingly, unlike the previous studies, the authors find that Eeryarestatin I does not induce ER stress, as indicated by the lack of CHOP activation in Eey1-treated cells, nor does it block autophagy. Instead, Eeryarestatin I induces DNA damage response. The authors propose that Eey1 may induce cancer cell death as a result of increased DNA damage.

Main points:

1. There is no evidence that DNA damage accumulation leads to cell death. The results presented are largely correlative in nature. Therefore, the authors cannot conclude that cell death is a result of DNA damage accumulation. The statement on page 7 (“we also determined ……”) and other similar statements throughout the paper should be toned down.

2. The authors conclude from Figure 3A that Eey1 induced apoptosis. However, I do not see any increase in the number of red dots in cells treated with 3µM Eey1 compared to control. Likewise, compared with Figure 4C, the authors conclude that there is an increase in the number of cells arrested in G1 phase, but the G0/G1 peaks in Figure 3C and D are very similar. They need to explain these experiments in a more clear way.

3. Unlike previous studies, the authors failed to detect ER stress in cells treated with Eey1. The interpretation of this negative result should be cautious because the authors do not have a positive control such as cells treated with those chemicals that normally induce ER stress regardless of cell types (tunicamycin etc).

4. How would DNA damage accumulation cause differential sensitivity to Eey1 in different cell lines? This point should be investigated by carefully comparing the level of DNA damage in cells sensitive to Eey1 to those more resistant cells PBMC.

Minor points:
1. There are already many different abbreviations for Eeyrestatin I in the literature, yet the authors come up with another one. This can easily confuse the readers.

2. Several papers have reported the anti-cancer activities of various p97 inhibitors. These papers should be cited.

**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