Reviewer's report

Title: Evaluation of bioactive sphingolipids in 4-HPR-resistant leukemia cells

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Reviewer: Riccardo Ghidoni

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The present manuscript shows that the known antitumor drug fenretinide is potentially dangerous in leukemia because of the occurrence of resistance mechanisms based on the accumulation of dihydroceramide and the consequent modulation of several derived sphingolipid metabolites. Moreover, even short fenretinide treatments, followed by drug wash out, lead to an acquired resistance although the sphingolipid pattern comes back to normal. The observation of the drug-presence dependency of phosphophosphorylated sphingosine, dihydrophosphinosine and dihydroceramide accumulation suggests that the signaling induced by those lipids could be crucial in the process of resistance acquisition. Indeed, impairment of dihydroceramide and ceramides glycosylation and dihydrophosphinosine phosphorylation is able to affect mitochondria activity of the resistant cells under HPR treatment.

I recommend the authors to pursue their studies and to investigate further the mechanism by which phosphorylated dihydrophosphinosine/sphingosine and dihydroceramide favors resistance acquisition (i.e. by DHcer desaturase over expression and ceramidase inhibition) as well as the connection between metabolic activity reduction of their resistant cells and the altered sphingolipid pattern. Moreover it would be very interesting, in such a model, to understand better the glucosylation events, first of all differentiating the dihydro and the unsaturated glucosyl and lactosyl ceramides formation.

Minor comments.

1. In the abstract: please list all the used chemotherapeutic agents and avoid the suspension marks.

2. In the methods section, please add one reference of the “XTT” mitochondria activity evaluation method.

3. In figure 1 legend. Please correct “cuadruplicates”.

3. In all the figures. Please remove the purple writing.

4. In figure 5 and 7. Please use comparable size for the graphs columns (B panels).

5. Discussion section, second page, last lane. Please invert “. [ref]” to the appropriate order.
Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Needs some language corrections before being published

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’