Reviewer's report

Title: Salinomycin increases chemosensitivity to the effects of doxorubicin in soft tissue sarcomas

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Reviewer: Ugo Testa

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The authors of this study have explored the sensitivity of three different sarcoma cell lines to salinomycin. The three different cell lines displayed a differential sensitivity to salinomycin in terms of cell growth inhibition. The addition of salinomycin in the presence of doxorubicin enhanced the anti-proliferative and apoptotic effects exerted by this drug. The authors have provided evidence through the analysis of the p53 signaling pathway that salinomycin sensitizes soft tissue sarcoma cells to the effects of doxorubicin, rather than inducing apoptosis by itself. The last section of the paper analyzes the effects at the level of the CD133+ cell compartment, showing that these cells are mainly sensitive to salinomycin and to the double drug treatment.

These observations are potentially interesting and support the potential use of salinomycin in experimental studies for sarcoma treatment. However, several of the observations raised in this study are too preliminary and need to be completed through additional experiments.

Specific Comments

- In order to better elucidate the occurrence of a possible synergism between salinomycin and doxorubicin the authors should perform experiments of quantification of living cells and of apoptotic cell number growing the cells in the presence of a dose-response of doxorubicin, either in the absence or in the presence of salinomycin (1uM). After that, they have to express the results in terms of percentage with respect to their respective control (the value of the control is 100%) and plot them on a X, Y graph. This is an intuitive way to directly visualize the occurrence or not of synergism. Furthermore, an isobologram analysis is important.

- Annexin V binding experiments would be useful in addition to the subG1 fraction analysis reported in Fig.2D.

- The data reported in Fig.4 provide very preliminary evidence that salinomycin affect the CD133+ cell population of the 1080 cell line. These results must be considered very preliminary. Given the high positivity of this cell line for CD133, it would be important to sort CD133+ and CD133- cell populations and then evaluate their sensitivity to salinomycin, doxorubicin and both drugs. Furthermore, in addition to vitality and apoptosis study, it would be particularly important to assess the effect of the two drugs at least on tumor sphere-forming capacity.
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'