Reviewer's report

Title: Potent inhibition of rhabdoid tumor cells by combination of flavopiridol and 4OH-Tamoxifen

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Reviewer: Magali Olivier

Reviewer's report:

In this manuscript, a new combination of treatment that can kill rhabdoid tumor cells is proposed. This type of tumors are very aggressive and have a poor prognosis (less than 15% 2-year survival rate). Because these tumors are currently treated with combinations of highly toxic chemotherapeutic agents which are poorly curative, there is a need to find new therapeutic regimens with better efficacy.

The authors have used three rhabdoid tumor cell lines to test the efficacy of combining flavopiridol and 4-hydroxy-tamoxifen (OHT) in killing these cells. The results show a potential benefit of combining flavopiridol and OHT to improve cell killing and provide some mechanistic data.

This study provide some interesting clues about a possible treatment strategy for rhabdoid tumors. However, most of the results are based on only one cell-line and the dose of OHT used is high. Furthermore some clarifications are required to make this manuscript fully convincing.

Major Compulsory Revisions:

1. While the rational of using flavopiridol come from a previous study where the authors have shown that flavopiridol, a pan-inhibitor of cdk, can induce cell cycle arrest and apoptosis in rhabdoid tumor cell lines and derived xenograft models, the rational for using OHT lack some explanations. Although the authors provide some background on the possible effects of OHT on cdk5s and cyclins, OHT is primarily targeting estrogen receptors and is used to treat cells expressing these receptors. Are the cells used in this study expressing these receptors? What is the role of these receptors in the biological effects reported in this manuscript? These points should be addressed and discussed.

2. The dose of OHT used in this study are very high. The molecular and cellular effects may be due to non-specific effects. This should be discussed and justified.

3. Data presented in figure 5 are not convincing. First, the induction of p21 by the combined treatment is not consistent between figure 5A and five C. In figure 5C, p21 is induced more strongly by flavopiridol than by OHT and the induction is the strongest by a combined treatment. However, in figure 5A p21 is less induced by a combined treatment (lane 8) than by flavopiridol alone (lane 1). Second, the
expression of Rb and cdks after single or combined treatments at different doses are quite confusing and poorly commented by the authors. I do not agree that OHT does not alter the effect of flavopiridol on the expression of cdks. In fact, OHT alone increases their expression and the results of combined treatments are difficult to interpret. How the authors explain the increase in Rb phosphorylation by 25nM flavopiridol?

4. In figure 6, please show the control experiment with Cy3 siRNA for cell-cycle and sub-G1 assays.

5. There are other inconsistencies in figure 7. The induction of caspase 2 and 3 activities at 12h presented in figure 7 D-E differ from the one presented in figure 7 J-K in terms of fold increase and effect of p53 silencing. Since it appears to be the same conditions, such variability would require a larger number of experiments and some statistical analyses to be fully convincing.

6. The authors have investigated the role of p53 in modulating the effects of these treatments on caspase activities and cell proliferation. They found that flavopiridol induces a p53-dependent G2 arrest that reduces the efficacy of flavopiridol or flavopiridol+OHT treatment. Since p53 is frequently inactivated by mutation in several types of cancers, the status of p53 in these cells should be determined, or it should be mentioned that p53 is indeed wild-type in these cells. Please also discuss the status of p53 in rhabdoid tumors. Moreover, it is concluded that the benefit of OHT addition is due to induction of a p53-independent apoptosis. However, p53 (and to a lesser extent p21) induction and G2 arrest are lower in flavopiridol+OHT compared to flavopiridol treatment (figures 4&5). Since tamoxifen has been reported to lower p53 responses (Guillot, 1996), it would be interesting to further assess p53 responses (regulation of p53 target genes involved in apoptosis vs apoptosis) in flavopiridol+OHT versus flavopiridol treated cells. A weaker induction of p53-dependent responses by flavopiridol in the presence of OHT may be responsible for the benefit of OHT co-treatment. Please comment.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests