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:

This revised version of the manuscript is improved and the authors have addressed many of the comments raised by both reviewers. In particular, major points 1 and 2 that I have raised have been satisfactorily addressed by the authors.

Nevertheless, some critical points remain to be better addressed to make this paper more convincing.

1. Effect of TAM alone.

The effect of TAM alone needs to be clarified at least in MON cells (add survival curve for MON+TAM). The authors state (p. 10) that “Increasing time of exposure to 4-OH-Tam alone did not result in enhanced inhibition in these cells (data not shown)”, however, 4-OH-Tam alone induced apoptosis more potently than flavopiridol alone as shown in figure 3B,C and figure 4B. 4-OH-Tam alone also induced caspase 3 activity more potently than flavopiridol alone.

I agree that combination of 4-OH-Tam and flavopiridol induce more apoptosis, but since 4OH-Tam induce more apoptosis, it is not clear if “4OH-Tam potentiates flavopiridol-mediated inhibition of RTs”, as stated in the conclusion, or if it is flavopiridol that potentiates TAM effect. Saying that “combination of flavopiridol and 4OH-Tamoxifen inhibit RT cells” as stated in the title is more appropriate. Flavopiridol and 4OH-Tamoxifen seem to have additive effects with TAM having more effect. Please show the effect of TAM alone in cytotoxic assays and clarify this point.

2. Quality and interpretation of figures 5 and 6B,C and role of cell cycle.

It is clear enough in the paper that either total or nuclear p21 was measured in the different figures. However, I am still not convinced by the data on p21 presented in figure 5. The pictures of p21 staining do not show a clear effect and the authors do not explain how they quantify these pictures to obtain data presented in figure 5C. Please add quantification method in the mat&met section.

It is clear from the data presented that flavopiridol induce a G2 arrest that is p53 dependent. However, the G2 arrest induced by flavopiridol+TAM shown in figure 4A is not reproduced in the new figure 6B added in this manuscript (control for p53 silencing experiment). It is thus not clear if TAM affect or not the flavopiridol-mediated G2 arrest. Moreover, the effect of p53 silencing on the
flavopiridol-mediated G2 arrest in the presence of TAM can not be evaluated. Thus, and as also pointed out by the other reviewer, the effect of cell cycle arrest on the cytotoxic effect of the combined treatments remain unclear. Data on apoptosis are more convincing. A suggestion would be to focus the paper on the results on apoptosis and caspases activities.

3. Lack of statistics in figure 7.

I agree that data presented in figure 7 show that flavopiridol and TAM increase caspase 3 and 9 activities with different kinetics and that combined treatment show stronger effects than either treatment alone. Although data presented in J,K show the specificity of caspase activity measurements, fold activities show some variations between different graphs. For example, in graph I at 12h time point, caspase 3 activity is increased by around 1.5 fold upon p53 silencing. In contrast, in graph K, the fold change is only around 1.2. A similar difference apply for the effect of p53 silencing on flavopiridol treatment alone (1.3 fold change in graph G and 1.8 fold change in graph K). Results from several experiments and statistics should thus be presented (especially graphs A-I) to strengthen these results.

4. p53 status in RT.

The authors indicate in the discussion that most RT have WT p53 based on two previous studies. However these studies seem to have analyzed p53 protein expression only, which is not representative of p53 gene status and protein function. One paper (Kinoshita, 2001) reported that 3/6 RT tumors carried p53 mutation. Discussion on p53 status in RT tumors should thus include this paper.

**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'