Reviewer's report

Title: Parallel Screening of FDA-Approved Antineoplastic Drugs for Identifying Sensitizers of TRAIL-Induced Apoptosis in Cancer Cells

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Reviewer: LANFENG DONG

Reviewer's report:

Major Compulsory Revisions:
The aim of the authors is to identify drugs that sensitize resistant cancer cells to TRAIL-induced apoptosis. They selected a group of candidate compounds from the FDA-approved anti-neoplastic drugs, which may in turn lead to the discovery of compounds with significant synergy with TRAIL to kill the resistant cancer cells, such findings maybe of clinical significance.

The authors show good understanding of the field of TRAIL-induced cancer cell death as well as the mechanisms of the TRAIL- resistance from cancer cells, which are documented in both the Introduction and Discussion parts of the manuscript. In general, the manuscript is written in a reasonably good way, even though it could be simplified and shortened, in particular regarding to the Method and Results parts. However, there is a disparity between the more theoretical parts of the manuscript and the actual experiments that were conducted and that are reported in the manuscript.

The major problem in this manuscript is that the authors actually emphasized apoptotic cell death in their manuscript, according to the title “Parallel Screening of FDA-Approved Anti-neoplastic Drugs for Identifying Sensitizers of TRAIL-Induced Apoptosis in Cancer Cells”, However the authors did not use any standard method to assess apoptosis induction/progression. The only assay they used is MTT assay which the colorimetric assay for measuring the viability and proliferation of cells and cannot be used for apoptosis testing. The authors need to use the proper methods to measure apoptosis, for example the annexin V assay using flow cytometry, the DNA fragmentation assay by Western blot assessed by agarose electrophoresis and/or to detect the cleaved forms of caspase-3 or PARP.

Minor Essential Revisions:
Below is the list of additional problematic points that need to be addressed:

1. Figure 1 shows results of treatment of PC3-TR cells with different concentrations of TRAIL for 24 h assessed by the MTT assay, Therefore the Y axis of the graph in Figure 1 should be the percentage of cell viability, not cell death. The same problem applies to other figures (6-10).

2. Figure 2 shows the experimental design of the drug testing, which is also explained in the text of the manuscript. Thus, Figure is redundant and should be
deleted.

3. The authors first pre-screened the selected drug candidates using a 20 #M concentration for each chemical, then 10##M as a lower concentration for sensitizing TRAIL-induced apoptosis. There must be a reason for the author to do so. However this strategy is unclear and should explain.

4. In pages 11, 12 and 13, the authors document the best combination treatment points of doxorubicin, mithramycin and mitoxantrone. In the related Figures (6, 7 and 8), an arrow should indicate the right point to make it clear.

5. There are too many figures in this manuscript showing the same type of testing using different drugs. The author should combine some of them, as they were generated using same testing method.

Discretionary Revisions:

Two candidates were shown to be promising to sensitize prostate and pancreatic cancer cells to TRAIL, being mitoxantrone and mithramycin. While this is interesting, it is purely observation. There are many compounds that sensitize cancer cells to TRAIL-induced the apoptosis. The interesting question is of understanding the mechanism, which is responsible. Therefore the author should address this issue to the two selected drugs, using the TRAIL-resistant and TRAIL-sensitive prostate and pancreatic cancer cells. There is a number of possibilities to study from this point of view, such as the role of the anti-apoptotic protein c-FLIP, activation of pro-apoptotic caspase-8, expression of the TRAIL receptors, etc

**Level of interest:** An article whose findings are important to those with closely related research interests

**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