Reviewer’s report

Title: RITA (Reactivating p53 and Inducing Tumor Apoptosis) is efficient against TP53 abnormal myeloma cells independently of the p53 pathway. A comparative study with nutlin3a.

Version: 2
Date: 26 March 2014
Reviewer: Manujendra Saha

Reviewer’s report:

In this revised manuscript the authors responded adequately to the reviewer’s criticisms by adding additional data and modifying the text. Although, the quality of the paper is improved in the revised version addressing the following minor issues will further strengthen the manuscript.

1. Based on the data presented in this manuscript, anti-myeloma activity of RITA can be best described as independent of p53 status. However, as mentioned by the authors, the role of p53 in RITA-induced apoptosis of MM cells can not be fully excluded (Page 11). This is due to the fact that although RITA can induce apoptosis in MM cells in the presence or absence of functional p53, genetic knockdown of p53 resulted in partial inhibition of apoptosis induction by RITA (Fig. 3). Importantly, p53 knockdown in H929 cells resulted in inhibition of the activation of Noxa, caspase-3 or caspase-9 (Fig. 3C) suggesting at least a partial role of p53 in this process. In addition, the statement in page 6 and Page 11 “RITA did not increase the expression of p53 targets” (especially in the subtitle) may be misleading to others since expression of p53 and its targets (Noxa, p21) were found to be either increased or decreased at some extent in some cell lines examined by the authors (Figure 2A). Therefore, the authors should describe the results in the abstract as well as in the text (page 6, 7, 11, and 12) taking consideration of the actual experimental observations as mentioned above.

Moreover, the title of the manuscript may also be revised. It would be better to say “……independent of p53 status…..” rather than “….p53 pathway…..” since this study did not provide enough evidence to support the claim that the effect of RITA is indeed independent of the p53 pathway.

2. It appears that both RITA and nutlin showed some toxicity (~30% killing) toward normal hematopoietic cells (PBMC) at higher doses of the drugs. This should be at least discussed in the discussion. Importantly, there are discrepancies for describing the assay methods in this particular experiment. As described in the “Abstract” and “Methods” sections, the apoptosis assay was performed with Apo2.7 staining of the cells. However, the figure shown for these results described and labelled the Annexin V positive cells (Fig. 1G) which have not been mentioned in the “Methods” section. Please clarify this.

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'