Author’s response to reviews

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.

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Author’s response to reviews: see over
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Dear Editor

We submit our revised article entitled “RITA (Reactivating p53 and Inducing Tumor Apoptosis) is very efficient against TP53abnormal myeloma cells independently of the p53 pathway, a comparative study with nutlin3a” by Surget et al for consideration for publication in BMC Cancer. In this second revised version, we provide the answers to all concerns raised by the reviewer, as detailed below. We hope that our revision article is now suitable for publication.

I certify that all authors reviewed the manuscript and have no conflict of interest to declare and that this work is not submitted in another journal and has not been previously published elsewhere.

Yours sincerely

Catherine Pellat-Deceunynck
Responses to Reviewer Manujendra Saha

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).

Response: the reviewer has analyzed the response to nutlin3a instead of to RITA. Sensitivity to RITA was not significantly modified upon p53 silencing in NCI-H929 (rather increased than decreased, anyway not significant), former Figure 3A left panel (now 3A). In contrast, sensitivity of H929 to nutlin3a was significantly decreased, former figure 3A right panel (now 3C). We have improved the figure legends to avoid any confusion.

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.

Response: This is the same fact as explained above. The western blot in former 3D (now Fig 3F) shows the p53 silencing effect in response to nutlin in H929 and not to RITA; indeed, p53 silencing inhibits Noxa and caspase activation induced by nutlin3a. By contrast, RITA-induced noxa increase or caspase activation was not inhibited upon p53 silencing: the western blot in former figure 3C (now 3E) shows p53 silencing effect in response to RITA in XG5 cells.

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.

Response: As suggested by the reviewer, we have made the following modifications
- abstract: The apoptosis that was induced by RITA was not related to the TP53 status of the cell lines or the del17p status of the primary samples (p=0.52 and p=0.80, respectively), and RITA did not commonly increase the expression level of p53 or p53 targets i.e., Noxa, p21, Bax or DR5, in sensitive cells.
- Subtitle page 6 “RITA did not increase the expression of p53 targets such as p21 and Bax”
- page 7: Except for DR5, expression of which was slightly increased in NCI-H929 only (1.26-fold increase), RITA failed to significantly increase the expression of p21 or Bax in sensitive TP53wt or TP53Abn cell lines but increased that of Noxa in all HMCls (Figure 2A). These results showed that an increase in expression of the p53 targets p21, Bax and DR5, was not a hallmark of cell response to RITA.
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.

Response: We disagree with this conclusion, which it is not supported by our results. The reviewer proposed this modification because of a confusion made between RITA and nutlin3a experiments in Figure 3, as mentioned above. As long as this matter is clarified now, we suggest to keep the title as it was originally presented, because it is supported by the results.

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 at least be 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

Response: as suggested by the reviewer, this point has been clarified in the Methods section and a sentence has been added in the discussion

_all modifications have been underlined in the manuscript._