Author's response to reviews

Title: PRIMA-1MET induces death in soft-tissue sarcomas cell independent of p53.

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Version: 2 Date: 26 June 2015

Author's response to reviews: see over
Reviewer's report

**Title:** Anti-tumor activity of PRIMA-1MET in soft-tissue sarcomas is independent of p53 and related to oxidative stress

**Version:** 1  
**Date:** 21 January 2015

**Reviewer:** M Joseph duffy

**Reviewer’s report:**
This manuscript investigates the anti-tumor potential of PRIMA-1MET in 5 STS cell lines and concludes that the compound “does not warrant further development as a TP-53 targeted therapy in this setting”. Rather, the authors conclude that PRIMA-1MET acts via inducing ROS toxicity. Overall, the manuscript is well written and the study is novel, being the first to investigate PRIMA-1MET in STS cells.

**Specific points**

**Abstract:** The conclusion that PRIMA-1MET does not act via p53 in STS cells is too strong based on the finding from one p53-null STS cell line and a small number of p53 WT cells, especially when considering the multiple published papers suggesting that this compound does act, at least in part, via mutant p53.

**Introduction:** This is concise and informative.

**Methodology:** This is well written.

**Results:**
1. Since the key conclusion from the STS cell lines largely result from one null cell line, it is essential to show that these cell are null for mutant p53 including its isoforms. This information may already be available in the authors lab
2. The authors state that PRIMA-1MET does not induce apoptosis but later add that it causes late apoptosis/necrosis in 3 mutated cell lines compared to the deleted and null cell line. What is meant by late apoptosis? The authors should elaborate on this.
3. What concentrations of PRIMA-1MET were found to induce autophagy?
4. The authors should show their data on PRIMA-1MET inducing JNK phosphorylation.

**Discussion:** In view of the multiple previously published papers showing that PRIMA-1MET can induce apoptosis in a variety of cell types, the authors should discuss possible reasons for their failure to observe this.

Points 1-4 above should be addressed.

**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 interest.
Reviewer's report

**Title:** Anti-tumor activity of PRIMA-1MET in soft-tissue sarcomas is independent of p53 and related to oxidative stress

**Version:** 1  
**Date:** 27 January 2015  
**Reviewer:** Staffan Strömblad

**Reviewer's report:**

**General comments**

This study aims to investigate the anti-tumor activity of the compound PRIMA-1-Met in sarcoma. Previous studies have identified p53-dependent and p53-independent mechanisms of action for PRIMA-1-MET, including the involvement of reactive oxygen species (ROS) in myeloma (Tessoulin et al 2014). While the present study investigates a different cell type (sarcoma), it does not address any novel mechanism of action. Still, this study falls short in comparison to the myeloma study in that the number of cell lines here studied is insufficient for the conclusions drawn and this study also present correlative evidence only, i.e. fails to address any functional aspects of the proposed mechanisms, although such claims are repeatedly made. I also find several other main conclusions not to being substantiated by the provided evidence and thus that this study is too preliminary for publication.

**Specific comments (Major compulsory revisions)**

The main conclusions of this study are not substantiated, including the title of the paper “Anti-tumor activity of PRIMA-1-MET in soft-tissue sarcomas is independent of p53 and related to oxidative stress”.

a) “Anti-tumor activity” has not been examined in this study, since the study is limited to cell lines. To substantiate this claim, authors need to perform testing on tumors in vivo.

b) The independence of p53 is based solely on correlative information limited to a few cell lines and therefore, this conclusion is based on a much too small sample size (p53 null cell: n=1; p53 wt cell: n=1; and p53 mutant cells: n=3). As PRIMA-1-MET has been reported to act through different mechanisms, the efficiency in a particular cell line will depend on several different factors regardless if it involves p53-dependence or not. Therefore, it is not possible to make safe conclusion based on n=1, but will require a statistically sound n. As a comparison, the above mentioned myeloma cell study included 27 human myeloma cell lines (HMCLs) and 23 primary samples. Alternatively, authors need to perturb p53 so as to examine the specific role in the same cell line with or without p53.

c) The claim of relation to oxidative stress is not properly addressed, since there is no functional evidence provided. Currently, also this part of the title appears misleading. In addition, the authors make firm conclusions in the text that the anti-tumor activity of PRIMA-1-MET in STS results mainly from off-target effects involving ROS-associated toxicity. To this end, authors need to substantiate their conclusions by perturbing the oxidative stress pathways to be able to make any functional link between the effect of PRIMA-1-MET and oxidative stress.

d) In the abstract as well as elsewhere, authors conclude that their results do not warrant further development of PRIMA-1MET as a TP53-targeted therapy in
sarcoma. However, the current clinical development of PRIMA-1-MET (APR246) is focused on the p53-dependent synergy between PRIMA-1-MET and chemotherapy, with ongoing clinical trials to this end. Given that the authors only tested the effect of PRIMA-1-MET as a monotherapy, their results are insufficient to warrant or not further clinical development on sarcoma. To substantiate their claim, authors therefore need to test the potential synergy effect between PRIMA-1MET and sarcoma-relevant chemotherapy in STS cells, including the potential p53-dependence of such synergies.

2. IC50 values should be calculated from the dose curves presented in Figure 1 and proper statistics applied to test if differences are statistically discernable. Likewise, proper statistical testing should be applied to all the quantifications presented in Figures 2-4. The quantification presented in Figure 4a needs to be performed among at least three experiments to facilitate proper statistical evaluation, and the same accounts for all the experiments in this paper; immunoblots, FACS data as well as immunofluorescence based localization all need to include proper quantifications and statistical evaluations.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:** I am a co-founder and minority owner of Aprea AB, a company that performs commercial development of PRIMA-1-MET. Additional knowledge on the mechanism of action of PRIMA-1-MET will be beneficial for Aprea AB in the clinical development and help them to direct this development.