**Author's response to reviews**

**Title:** Smac Mimetic-Derived Augmentation of Chemotherapeutic Response in Experimental Pancreatic Cancer

**Authors:**

- Niranjan Awasthi (niranjan.awasthi@utsouthwestern.edu)
- Amanda Kirane (amanda.robinson@utsouthwestern.edu)
- Margaret A Schwarz (margaret.schwarz@utsouthwestern.edu)
- Jason E Toombs (jason.toombs@utsouthwestern.edu)
- Rolf A Brekken (rolf.brekken@utsouthwestern.edu)
- Roderich E Schwarz (roderich.schwarz@utsouthwestern.edu)

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Re: Revised manuscript submission, MS: 5449271380908824

Dear Miss Judith Gorton:

Thank you for the review of the manuscript “Smac Mimetic-Derived Augmentation of Chemotherapeutic Response in Experimental Pancreatic Cancer” by Awasthi et al., submitted earlier for publication in BMC Cancer. We would like to thank the two reviewers for their insightful and detailed comments. Thank you for granting some additional time until this resubmission of the revised manuscript.

In the revised manuscript we have made required changes (highlighted in blue) according to the reviewer’s suggestions. All comments made by the reviewers have been addressed individually, as stated in detail as follows:

Reviewer- 1

Comment 1. The abstract should be written in a more concise way. The inclusion of drug concentrations seems to be too tedious for an Abstract.

Response. As suggested by the reviewer, we have now rewritten abstract more concisely. The abstract word count is now 236 as compared to 296 previously.

Comment 2. Similar results have been published and cited in the manuscript (Dineen et al. 2010). It would be nice to make some comparisons between two studies in the Discussion.
Response. We have included a paragraph in the “Discussion” section and provided a more detailed comparison between two studies. Please see Page 13.

Comment 3. The significance of the paper could be improved if it were provided with biochemical analysis on mouse tumor tissues.

Response. We agree with the reviewer that this would be interesting, however, the objective of our current study was to extend previous tissue-based and survival related JP-gemcitabine combination effects as seen in an orthotopic model (Dineen et al. 2010) into a more generally applicable use: to evaluate potentially clinically significant in vivo treatment effects of that combination in other cell lines, and to examine the ability to combine JP with other cytotoxic agents beyond gemcitabine. Since several of these combinations have shown a survival benefit to combination therapy, and since we only performed a small-scale local tumor growth experiment with a different cell line, we did not expect a mere tissue based analysis of apoptotic index or cell proliferation within this tissue to add significantly to the conclusions reached from our experiments.

Comment 4. There are some discrepancies in the results from the animal survival experiments (Fig. 6A and 6B.) For instance, for single JP treatments, the survival time was 28 d for Fig. 6A, but 21d for Fig. 6B. What caused the large variability?

Response. This was an oversight and we thank reviewer for pointing this out. Actually, survival study presented in Figure 6A animals received maintenance therapy while animals in study 6B received 2 weeks therapy. This might be the reason for longer survival of animals in single JP treatment group in Figure 6A compared with study presented in Figure 6B. This possible explanation was added to the manuscript in the methods and results sections. In addition, some variability between individual in vivo experiments exist, and may possibly contribute to the differences observed.

Comment 5. Although Annexin V staining showed that GEM had an effect on early apoptosis (Fig. 2A), the PARP-1 cleavage experiment did not detect any cleaved PARP-1 (Fig. 2B). Please provide an explanation on this. In addition, a loading control for Fig. 2B could be helpful.

Response. Annexin V staining is an early marker of apoptotic cells whereas PARP-1 cleavage represents a late event in the apoptotic cascade that is executed specifically by caspases. In our study we observed that Gem treatment of AsPC-1 cells for 12 hours induced Annexin V positive cells, however no PARP-1 cleavage was observed after Gem treatment for 24 hours. This is probably because of short incubation (24 hours) of AsPC-1 cells with Gem that is not enough to cause detectable levels of PARP-1 cleavage. This explanation is supported by some studies in the literature that Gem treatment may require 48 to 72 hour incubation to get detectable levels of PARP-1 cleavage (please see the five reference listed below). We have now included this explanation in the “Discussion” section.

We have also included a loading control blot picture in Figure 2B and Figure 4 as
suggested by the reviewer.

References:

Reviewer - 2

Major Comment 1. The most critical study (Fig. 5) was conducted with a very small number of mice. This is the crucial weakness of this paper. If the authors could repeat the same experiment using at least 6-7 mice per group and obtain identical results, this would be meaningful.

Response. As mentioned above, the objective of our current study was to extend previous tissue-based and survival related JP-gemcitabine combination effects as seen in an orthotopic model (Dineen et al. 2010) into a more generally applicable use: to evaluate potentially clinically significant in vivo treatment effects of that combination in other cell lines, and to examine the ability to combine JP with other cytotoxic agents beyond gemcitabine. Since several of these combinations have shown a survival benefit to combination therapy, and to local tumor growth in a subcutaneous model, all of which had at least six animals per treatment group (in some groups up to eight), we only performed a small-scale orthotopic local tumor growth experiment with a different cell line for added validation. These models are rather resource-intensive and somewhat expensive, and we think that the results from our other in vivo experiments are sufficiently valid even without larger numbers of animals per group in this single orthotopic experiment. Furthermore, the previously mentioned orthotopic model paper (Dineen et al., 2010) showing a JP combination therapy benefit was based on four animals per group. We have therefore decided against additional in vivo experimentation in this single area.

Minor Comment 1. For the Western blot analysis (Figs. 2B and 4), loading control such as actin or GAPDH is generally presented. It is difficult to evaluate if higher
expression is indeed due to high protein expression or larger amount of sample application without a loading control.

Response. We agree with the reviewer’s suggestion, and have now provided Western blot analysis for alpha-tubulin as loading control for Figures 2B and 4.

Minor Comment 2. There are several typographical spacing problems at scattered places throughout the manuscript.

Response. We have fixed all typographical spacing problems throughout the manuscript.


Response. As suggested by reviewer, we have italicized “in vivo” and “in vitro” throughout the manuscript.

We hope that the revised manuscript now meets all requirements for successful acceptance, and we believe that the readers of BMC Cancer will find this a very useful contribution.

Thank you very much for your interest.

Sincerely yours,

Roderich E. Schwarz, M.D., Ph.D.
Chair, Surgical Oncology
Department of Surgery
UT Southwestern Medical Center
5323 Harry Hines Blvd.
Dallas, TX 75390-8548
Tel.: (214) 648-5865; Fax.: (214) 648-1118; Email: Roderich.Schwarz@utsouthwestern.edu