Author's response to reviews

Title: Differential modulation of nicotine-induced gemcitabine resistance by GABA receptor agonists in pancreatic cancer xenografts and in vitro

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Author's response to reviews: see over
Dear Editor:
We have made substantial revisions to our manuscript in response to critique by the reviewers and hope that our manuscript will now be accepted for publication.

Responses to reviewer Amir Avan
Major compulsory revisions:
1) Regulations by our IACUC committee mandate that the mice be sacrificed as soon as xenografts have reached a diameter of 1.5 cm. Since xenografts from BXPC-3 cells reach that diameter within 4 weeks of subcutaneous injection, we had to start all treatments a day after injection of cancer cells in order to ensure a 4 weeks duration of treatments.
2) We have expanded our discussion to address reviewer’s query (lines 12 – 16 on page 18).
3) The abstract, discussion and conclusions have been changed in accordance with reviewer’s suggestions (page 2, line 20; page 3, lines 9-11; page 18, lines 21, 22; page 22, lines 8-10).

Minor essential revisions:
In accord with critique by reviewer Dr. Chellappan, the statistics results have been added to the figures and figure legends. In order to make the results easier to read, we have therefore taken the statistical details out of the results section, thereby eliminating the repetitive wording criticized by this reviewer.

Figure 1A, B: We have not included photographs of tumor bearing mice because such illustrations in a previous publication triggered death threats to Dr. Schuller from animal rights activists. Dr. Schuller truly does not wish to repeat that experience! We also believe that the graphs of xenograft volumes are more informative than photographs because they show quantitative changes that have actually been measured.

Responses to reviewer Davide Melisi
Major compulsory revisions:
Quantitation of data and statistical analyses have been added as requested (see revised figures 1-4 and 6, new figures 5B, 7B, 7C, 8B 8C).

Modulation of survival times by treatments could not be investigated because IACUC forced us to euthanize for “humane reasons” all mice when their xenografts had reached a diameter of 1.5 cm. This was the case in the control group 4 weeks after subcutaneous injection of the tumor cells. As we needed mechanistic data collected from xenograft tissues at an identical time point to allow for comparisons among different treatment groups with controls, all animals were euthanized at that time.
In previous figure 5 (now figure 6A) the legend for the x-axis has been revised as requested.

Responses to reviewer Srikumar Chellappan

Major essential revisions:
IACUC regulations required us to euthanize the mice as soon as one diameter of their xenografts had reached 1.5 cm. This was the case in the animals of the control group 4 weeks after subcutaneous injection of the tumor cells. Since comparison of functional data (cAMP, signaling proteins, caspase-3 and EGR-1) among controls and treatment groups requires that the data are collected at the same time interval, the entire experiment had to be terminated at that time. Data from additional time points can therefore unfortunately not be generated.

Details of statistical analyses by ANOVA and post-tests has been added to the graphs and/or figure legends as requested (revised figures 1-4 and 6A) and additional quantitative densitometry was generated from triplicate western blots (new figures 5B, 7B and C and 8 B and C).
The discussion (page 18, lines 21, 22) and conclusions (page 22, lines 9, 10)

We have made every possible effort to make the changes requested by the reviewers and hope that our paper will now be rapidly published.

Sincerely,

Hildegard M. Schuller, DVM, Ph.D.
Distinguished Professor of Comparative Pathology