Author’s response to reviews

Title: Phosphatidylinositol 3-kinase inhibitor induces chemosensitivity to a novel derivative of doxorubicin, AD198 chemotherapy in human bladder cancer cells in vitro

Authors:
Dmitriy Smolensky (dsmolens@vols.utk.edu)
Kusum Rathore (krathore@utk.edu)
Maria Cekanova (mcekanov@utk.edu)

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Author’s response to reviews:

Response to REVIEWER #1: Accept after minor essential revisions

MC: We thank for your support to publish our study. We have made several minor revisions to improve this manuscript.

Response to REVIEWER #3: Accept without revision

MC: We thank for reviewer’s support to publish our study.

Response to REVIEWER #4: Major revisions required

MC: We have not received any specific questions or comments from the reviewer#4, so we were not sure how to address his/her comment. We have made several minor revisions to improve this manuscript. To strength our findings, we referenced also our recently published study where we validated AD198 superior inhibition of cell growth in canine bladder and osteosarcoma cell lines over Dox in vitro.

Response to REVIEWER #5: Major revisions required

The work by Dmitriy et al., is an attempt to to compare the efficacy of Dox and AD198 on human bladder cancer and explore their mechanisms in inhibition of the human bladder cancer cells in vitro. They used 2 human transitional cell carcinoma (TCC) cell lines, T24 and UMUC-3, to make their point. Although the results exhibit the similar mechanism of action for both the drugs, the efficacy of in modulating the various cellular events like cell survival, ROS generation, apoptosis and MAPK pathways are variable. The results showed that AD198 is superior to Dox in promoting cell survival and ROS generation whereas Dox is better in inducing
apoptosis and phosphorylation of AKT1. I also noticed a variable behaviour of the drugs in two cell lines. Additionally, I will suggest to authors to delete the following sentence or include the appropriate data/reference for it. "AD198 might act through different apoptotic mechanisms"

MC: We have also added several additional reference (REF#25, 4142 56-58) to discuss and compare our findings with previously published studies in more details. See modified Discussion on pages 12-15 (third and fourth paragraphs of Discussion section). The difference in mechanism of their action is due to different location, targets, and activation of signaling pathways in cells. In addition to ROS production in the cytoplasm, Dox induces DNA damage via Topoisomerase II, while AD198 mainly functions in the cytoplasm by increasing ROS and activating PKC-δ (REF 40). Our data correlates with the previously published study confirming that AD198 and Dox may induce apoptosis in caspase-dependent and -independent pathways. Our results showed that AD198 induced cytoplasmic ROS production more as compared to Dox, even Dox had shown increased caspase signaling pathway due to increased possible DNA damage as compared to AD198.

We agree, human cell lines that we used as model for in vitro experiments to evaluate efficacy of novel therapy have some limitations. We need to take into considerations that currently used human established cells lines have mutation of different genes and we need to take into our final validation. We have tested additional three canine primary bladder cancer cells to compare efficacy of AD198 and Dox in additional cell lines to make sure that we cover more spectrum of cell lines. Our study utilizing canine cell lines three bladder and three osteosarcomas that were recently published in Journal of Drug Design, Development and Therapy (Ref#25) confirmed that AD198 is more effective in inhibition of cell proliferation and inducing apoptosis in canine TCC and osteosarcoma primary cell lines than Dox through the p38 MAPK signaling pathway.

In my view, although the findings reveal that AD198 could be effective in the treatment of bladder cancer, the study can't be considered conclusive without in vivo studies. Additionally, the inclusion of animal data will illustrate the clear picture and will rule out the confusion due to variable results in cell lines.

MC: The in vivo experiments will be next logical step to prove safety and efficacy of AD198 in bladder cancer that we detected in vitro, however in vivo experiments were not in the scope of this manuscript.