Author's response to reviews

Title: Therapeutic effect of intravesical administration of paclitaxel solubilized with poly(2-methacryloyloxyethyl phosphorylcholine-co-n-butyl methacrylate) in an orthotopic bladder cancer model

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Author's response to reviews: see over
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Dr. Ryan M. Relox
Editorial Office ‘BMC cancer’

Dear Dr. Relox

We wish to thank you and the members of the Editorial Board for the helpful comments on our manuscript entitled “Therapeutic effect of intravesical administration of paclitaxel solubilized with poly(2-methacryloyloxyethyl phosphorylcholine-co-n-butyl methacrylate) in an orthotopic bladder cancer model” (MS:1028495963139995).

We have carefully reviewed the comments of the reviewer and have addressed each concern in the attached response letter. We additionally have made changes to our manuscript to adequately address the comments of the reviewer. At the same time, we have added another author in the revised version because we found one of authors is missing in the previous version.

The changes to the manuscript are indicated in the text using track changes.

We hope we have now satisfactorily completed the manuscript such that it will now be acceptable for publication in the BMC Cancer.

Sincerely,

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Responses to comments of Reviewer 1)

We are grateful to Reviewer 1 for the critical comments and useful suggestions that have helped us to improve our manuscript considerably. As indicated below, we have taken all these comments and suggestions into account in the revised version of our manuscript.

1. The authors address an important question in non-muscle invasive bladder cancer therapy. New approaches are needed in the studies performed in this preclinical model system are a first step in moving toward validation of new therapeutics for non-muscle invasive bladder cancer.

Response: We thank Reviewer 1 for the helpful comment. We face a critical issue with respect to the lack of new treatment modalities of bladder cancer, especially for BCG failure cases. We previously evaluated the characteristics of BCG refractory[1], as well as BCG relapsing[2] non-muscle invasive bladder tumors in our clinical dataset and found their high malignant potential for stage progression and ultimately for cancer-related death.

   In our laboratory, we have tried to establish a new therapeutic modality for bladder cancer including the new combination chemotherapeutic of S-1 with CPT-11[3], the metastatic suppressor peptide metastatin[4], the novel NF-κB inhibitor DHMEQ[5], and the AT1R antagonist candesartan[6]. We also investigated the therapeutic effects of intravesical instillation of interleukin-15 gene therapy[7] using the same in vivo MBT-2 orthotopic bladder cancer model for the purpose of the establishment of new intravesical therapy for non-muscle invasive bladder cancer. In the present study, we propose a new drug delivery approach using novel nanoparticles which enable high drug concentrations in tumors.

References)

110(11 Pt B):E508-513.


2. The data are intriguing that additional information is needed to confirm what this reviewer perceives to be preliminary observations. It is important for the authors to define the number of experiments and the reproducibility of the data that are presented in figures 1 through 3. The data should be reproducible in at least 3 independent experiments.

Response: Firstly, as for *in vitro* experiments evaluating the cytotoxicity of vehicle, we performed the LDH assay in three independent sets of experiments and similar results were observed in these three sets of experiments (another 2 sets of experiment are shown in Figures A1 and A2 below).

Figure A1
Furthermore, the cytotoxicity of the vehicle was also evaluated using the WST assay, which is another cytotoxic assay. In the WST assay, Cremophor exhibited dose-dependent cytotoxicity towards MBT-2 cells, whereas no cytotoxicity was observed with PMB30W (as shown in Figure B below).
Thus, we confirmed the cytotoxic effect of Cremophor but not in PMB30W with reproducibility in the LDH and WST assays. We added the sentence, “The experiments were repeated independently three times.” in the Methods section (page 8 line 7) and Figure legends of Figure 1 (page 20 line 24) in the revised manuscript.

Secondly, we tried several sets of such in vivo experiments previously. At first, we tried to administer PTX-30W less than 6 times and sacrificed the mice at a later time point, however, it did not work properly. Finally, we set the PTX-30W administration schedule as shown in the original manuscript. Another set of in vivo experiments demonstrated that intravesical administration of PTX-30W resulted in a significant reduction of bladder wet weight (54±12 mg; n=7) as compared with those of the control group (111±21 mg; n=6, p=0.0370) and PTX-CrEL group (139±34 mg; n=6, p=0.0450). There was no significant difference between the control group and PTX-CrEL group (as shown in Figure C below).

Figure C
Lastly, for the paclitaxel uptake study presented in Figure 3 in the original manuscript, we carried out this uptake study only once, however, the paclitaxel uptake of PTX-30W and PTX-CrEL was measured in 12 mice and 10 mice, respectively. We feel this number is large enough to compensate for the lack of reproducibility. In a previous study evaluating the efficacy of intraperitoneally administered PTX-30W in a peritoneal metastasis model of gastric cancer in nude mice, intratumoral concentrations of paclitaxel were significantly higher in the mice administered PTX-30W as compared with those administered PTX-CrEL [8]. Although the route of administration differed from that of our present study, their results also suggest superior uptake of paclitaxel by PTX-30W into the tumor site when it was administered intracavitarily.

Reference)

3. The data shown in figure 3 should be evaluated for impact on antitumor activity. The concentration of drug delivered should be correlated with tumor size to determine if there is a direct relationship between drug delivery and the antitumor response.

Response: We agree with the comment of Reviewer 1 that the concentration of the drug should be correlated with tumor size. We appreciate the comment, but this is
one of the limitations of the present study. What we wanted to determine in the
paclitaxel uptake experiment in bladder tumors (Figure 3 in the original
manuscript) was whether or not just one instillation of PTX-30W could deliver
enough paclitaxel to the bladder tumor (on day 20). Although this is a limitation,
we believe that a high drug concentration after single instillation influenced the
antitumor activity of the drug. We discuss the limitation and added the sentences
“One limitation of the present study is that we did not evaluate the correlation of
the tumor concentration of PTX-30W with tumor size. However, we believe that a
high drug concentration after a single instillation of PTX-30W had an effect on the
antitumor activity of the drug.” in the Discussion section in the revised manuscript
(page 16, line 1).

4. It also will be important to establish the reproducibility of drug incorporation in the
mixture. Data should be provided to demonstrate that separate lots are comparable.

Response: In this study, we performed these experiments using different lots of
Cremophor and paclitaxel (in Figure A1 and A2 shown above). Different media
were made up for each experiment. In the experiment in Figure 1 in the original
manuscript, the lot numbers of Cremophor and paclitaxel were KPF0963 and
STF2531, respectively. The lot numbers of Cremophor and paclitaxel were
AWE0963 and CDE2103 in Figure A1 and TLH0963 and STF2533 in Figure A2.

One small editorial note - Cremophor is abbreviated PTX–Cre. This abbreviation should
be modified as it contains the abbreviation (Cre) that designates an enzyme used in lox
P systems to genetically modify gene expression.

Response: We are grateful for the comment and accordingly have revised the
abbreviation “PTX-Cre” to “PTX-CrEL” in the revised manuscript. The abbreviation “CrEL” stands for Cremophor® EL.
Response to the reviewer 2’s comments)

We are grateful to Reviewer 2 for the encouraging comments and appreciate the effort to review our manuscript.

5. Results: The authors show less cytotoxicity to MBT2 cells when exposed to PTX-30W. In vivo, bladder weights were decreased and tumors showed increased uptake by liquid chromatography. Multiple apoptosis assays are commercially available. Do any others demonstrate apoptosis?

Response: We did not investigate the effects of Cremophor and PMB30W on apoptosis. We investigated the cytotoxic effects in different ways using the LDH assay as well as the WST assay on several occasions. As shown in Figure B below, Cremophor exhibited dose-dependent cytotoxicity towards MBT-2 cells, whereas no cytotoxicity was observed with PMB30W in the WST-assay.

Figure B