Reviewer 4

pen Review

(x) I would not like to sign my review report

( ) I would like to sign my review report

English language and style

( ) Extensive editing of English language and style required

(x) Moderate English changes required

( ) English language and style are fine/minor spell check required

( ) I don't feel qualified to judge about the English language and style

	Yes	Can be improved	Must be improved	Not applicable
Does the introduction provide sufficient ackground and include all relevant references?	(x)	( )	( )	( )
Are all the cited references relevant to the research?	(x)	( )	( )	( )
Is the research design appropriate?	( )	(x)	( )	( )
Are the methods adequately described?	(x)	( )	( )	( )
Are the results clearly presented?	(x)	( )	( )	( )
Are the conclusions supported by the results?	( )	( )	(x)	( )

Comments and Suggestions for Authors

The manuscript is well addressed.

Minor comments:

Fig 2C should be quantified and P-Value should be indicated

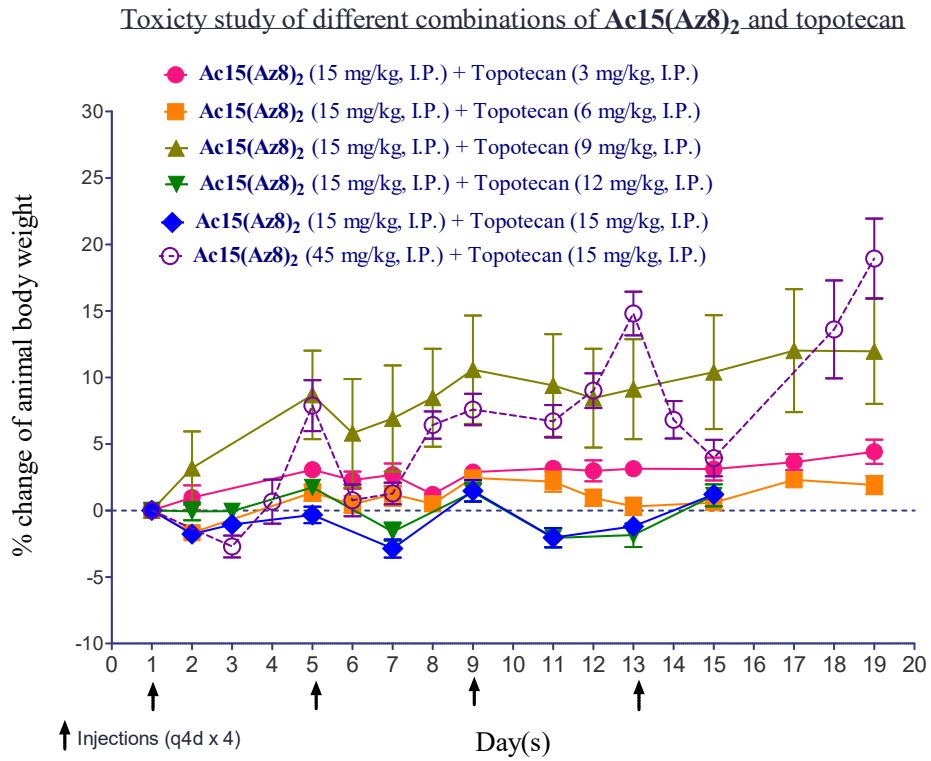
Reply: Fig 2C was an immunostaining result (Figure 3 now), we would like to demonstrate the localization of BCRP at the plasma membrane of S1M180, but not wild type S1. Immuno-staining is a qualitative approach and difficult to precisely quantify the DOX level in S1 and S1M180 cells because there were very few cells per fluorescent image. In addition to using fluorescence microscopy, we have quantified the intracellular DOX levels of S1 and S1M180 using fluorescence microplate reader (page 5, Figure 2B) which can be quantified and analyzed statistically.

Fig 6C, D and G addition of the Mice survival graph will be more relevant to the clinical point of view.

Reply: Thanks for your comments. We agree with the reviewer that mice survival graph is important and more relevant to the clinical point of view. There was no animal death in the three treatment groups during the in vivo efficacy studies. The mice in solvent control groups were terminated on days 55 because of the tumor overgrowth. Mice in topotecan treatment group and co-treatment group were terminated on days 71 because of the end of animal studies. We have included the number of animal deaths result in figure 8D (page 10).

Please comment on the toxicity of the drugs. Has it been tested for ?

Reply: **Ac15(Az8)$_2$** alone has no toxicity when it was dosed at 45 mg/kg (I.P.) every other days for 24 injections. Before in vivo efficacy study, we studied the toxicity of the combination effect of **Ac15(Az8)$_2$** and topotecan towards the Balb/c mice. It was found that all combinations at different dosages as indicated in the figure below were safe and induced no body weight loss and no animal death after 4 administrations of co-treatment (shown in the figure below). Finally, we chose 45 mg/kg of **Ac15(Az8)$_2$** (I.P.) and 15 mg/kg of topotecan (I.P.) to be our dosing amount in the in vivo efficacy study.



Submission Date

20 September 2022

Date of this review

16 Oct 2022 06:15:39