Reviewer’s report

**Title:** Molecular targets for the protodynamic action of cis-urocanic acid in human bladder carcinoma cells

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**Reviewer:** Tapas Saha

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Comments to the Author

This manuscript presents data on the photodynamic action of cis urocanic acid in human bladder carcinoma cells. The authors have a long history of publishing with this compound. The effects of cis-UCA are commendable to inhibit the cell proliferation and act as an anticancer agent against bladder cancer. However, there are some minor and major points that is described below.

1. The correlation between the actions of cis-UCA and its actual potential implications in clinical intravesical chemotherapy against bladder cancer is not well understood.

2. Why this will be specific for only non muscle bladder cancer. What about other types of bladder cancers.

3. One major concern is that Cis-UCA is not specific to the tumor cells. It will affect the bystander normal healthy cells as well so how they will be effective?

4. For Cell cycle analysis you do not need to synchronize the cells. It would have been better to show cell cycle arrest by treating asynchronous cells with the metabolite at different concentration.

5. The reason for recovery period after treatment was not explained and it also defers in hours. In one case it is 20 hours and in other case it is 24 hours. Which one is the correct number?

6. Cytoplasmic DNA –histone complex was monitored and it is shown in both the pH although it was written on the text that for ph 7.4 ‘it was not shown’. What are the consequences of cell released DNA-histone complexes with the anti-tumorigenic effects of cis UCA.

7. Preparation of cis-UCA is not given in the text. What is the function of PIPES? Is it a solvent for cis-UCA, if not then where is the vehicle control for the treatments?

8. cis-UCA both promote apoptotic and necrotic death of the cells at the same pace so how it is specific for inducing apoptosis in bladder cells.

9. I am not sure how much will be the efficiency of this compound for its photodynamics actions with so little change in pH (0.8)

10. Fig 2A demonstrates that at 4% cis-UCA the total cytoplasmic DNA histone complex was reduced when compared with 2%. I would like to see the effects on
the other parameters also at 4%. Decreasing the effects is a general phenomenon or it specific to the histone-DNA complex formation.

11. Explain the decrease in G0/G1 from the normal at 1% and then again it increased showing arrest in Fig 3. Error bars are reasonable and it looks like significant to this reviewer. Figure 2 demonstrates 3 folds increase in Caspase III positive cells (apoptotic activity) when compared to no treatment. Sub G0 portion of Figure 3 actually demonstrate apoptotic cells and this region is not matching with Figure 2. No effect on S phase. This is strange.

12. Figure 5, B and C looks exactly the same. Please write the substrates on the plot itself to differentiate it.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
'I declare that I have no competing interests'