Reviewer’s report

Title: Rho-kinase inhibitor HA-1077 suppresses the proliferation/migration and induces apoptosis of urothelial cancer cells

Version: 1 Date: 16 December 2013

Reviewer: Bharat Joshi

Reviewer’s report:

Major Comments:
1. Effectiveness of HA-1077 is tested in vitro in two bladder cell lines in terms of cell growth inhibition and cell migration. The drug also induces apoptosis in treated cells at different concentrations. It is not shown that this drug will be effective equally well in vivo animal model of bladder cancers developed by either UM-UC-3 or 5637 bladder cancer cell lines. It is known that these tumor cell lines are tumorigenic in experimental mouse model and also form colonies in vitro. The authors should test the effectiveness of HA-1077 and excipient control in vivo by developing an in vivo mouse model of human bladder cancer model. Either of the cells line can form subcutaneous tumors quickly in athymic nude mice as well as colonies in a clonogenic assay in vitro.

2. In Results section, the authors have shown IHC data for RhoA, ROCK-I and ROCK-II protein levels after HA-1077 treatment to the tumor cells. They should evaluate the extent of immunostaining (intensity of staining as 1+ through 3+) and score % positive score of treated cell line at each concentration of the drug.

3. Similarly, the authors should also describe the statistical analysis in Results section for each set of experiment.

Minor Comments:
3. The authors should look into the paragraph “Inhibition of cell growth” of Materials and Methods section for MTT concentration and volume added in each well. The manuscript reads 10 ml/well. This typo should be fixed.

4. In Discussion section, authors’ first ten sentences does not connect to the manuscript. Similarly, the entire third paragraph on the following page (which begins with “It is important to study……………..”) should be abridge in few lines. It is a disconnect in the present form of the manuscript.

5. The last paragraph of Discussion section is a disconnect and the authors should look into their references 24-29, which demonstrate that this drug is tested in clinical trials for patients with cardiovascular diseases. Perhaps, these references may be useful to study the safety and potency of the drug in preclinical mice model of bladder cancer as well as designing a clinical study in human patients with bladder cancer.
6. Some references are redundant and do not add any additional information.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**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'