Reviewer's report:

**Title:** Antitumor activities of ATP-competitive inhibitors of mTOR in colon cancer cells

**Version:** 2  **Date:** 17 November 2011

**Reviewer:** Qing-Bai She

The authors show in this manuscript that the mTOR kinase inhibitors (PP242 and NVP-BEZ235) are more effective than rapamycin in suppression of colon cancer cell growth in vitro and in vivo. This phenomenon has been demonstrated in various papers (PLoS Biology, 2009, 7(2):e38; Cancer Cell, 2010, 17:249–261; Cancer Res, 2010, 70(1):288-298; Mol Cancer Ther, 2011, 10(8):1395-1406). Thus, further mechanistic work is required to strengthen the conclusions drawn in this study.

**Major Compulsory Revisions:**

- It's not clear why the three colon cancer cell lines (LS174T, DLD-1 and SW480) are particularly chosen in this study. A large panel of colon cancer cell lines is needed to confirm the sensitivity in response to the treatment of PP242 and rapamycin. In addition, do PP242 and NVP-BEZ235 also effectively inhibit DLD-1 xenograft tumor growth as they did in LS174T and SW480 (Fig. 3)?
- PP242 can effectively inhibit both mTORC1 and mTORC2 at 100nM as evidenced by the dephosphorylation of S6 and AKT, respectively (Fig. 1), but why did authors use 10 µM of PP242 and 1 µM of NVP-BEZ235 in the cell proliferation and survival studies (page 5, line 4 from bottom)? Using 10 µM of PP242 may cause off targets such as to PI3K, PKC and others (PLoS Biology, 2009, 7(2):e38).
- A recently published paper (PLoS One, 2011, 6(9):e25132) showed that NVP-BEZ235 (500 nM) inhibits cell proliferation but has no effect on apoptosis as determined by cleaved PARP in DLD-1 and SW480 cells, whereas authors showed in the Fig.2 that NVP-BEZ235 (1 µM) induced apoptosis in the same two cell lines as assessed by a cell death detection ELISA. Is the difference because of off target and/or using different methods for the detection? It's better to combine other methodologies such as FACS analysis to confirm this difference.
- In Fig. 4, what is the mechanism underlying the synergistical antitumor activity by combination of the mTOR kinase inhibitors (PP242 or NVP-BEZ235) and the MEK inhibitor (UO126) regardless of whether mTOR inhibitors increase pMAPK? Can UO126 effectively inhibit pMAPK in vivo? Does the combination of both drugs inhibit cell proliferation and induce apoptosis in vivo as evidenced, for instance, by Ki-67, cleaved PARP or TUNEL?

**Minor Essential Revisions:**
• The references are needed for the preparation of PP242, NVP-BEZ235, rapamycin and UO126 in in vivo study (page 5, line 10 from bottom).
• In Fig. 1, one of cell lines is indicated as SW620, which should be corrected to SW480.

**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