Reviewer’s report

**Title:** Anti-tumor effect of bisphosphonate (YM529) on non-small cell lung cancer

**Version:** 1  **Date:** 5 October 2006

**Reviewer:** Takeshi Yuasa

**Reviewer’s report:**

**General**

In the present study, authors investigated the effects of YM529 in preventing the growth of non-small cell lung cancer (NSCLC) cells in vitro. In vitro, YM529 dose-dependently inhibited the growth of different bladder cancer cell lines possibly through the inhibition of phospho-ERK1/2. In addition, YM529 showed direct anti-tumor effect on NSCLC. The authors conclude that YM529 may be a potent anti-cancer agent for NSCLC. I have several comments.

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**Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)**

Authors show the growth inhibitory effect of YM529 against NSCLC cells. However, many previous reports have already demonstrated similar results to this manuscript using various types of cell lines. There seems to be little additional and important information. Moreover, these experiments are immature. Although authors did not demonstrate any Ras-contribution, authors concluded this growth inhibitory effect is due to the reduction of Ras-signal.

Moreover, it is critical to know whether the levels that were achieved in these NSCLC cells in vitro are ever likely to be achievable in humans. In order to let the readers to better understand their speculation that YM529 is useful for the patients with NSCLC, authors should show the growth inhibitory effect against the tumor bearing mice in vivo. I think this is the initial step for the translational research.

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**Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)**

1 As opposed to what is claimed in the introduction section (page 5), a previous study has demonstrated that ZOL reduced tumor growth of human squamous carcinoma in the tibiae of treated mice (Tannehill-Gregg SH et al., 2006). The authors should discuss this previous study along with their own data.

2 page 4 and page 11: what is the R2 ? Although it might be a common word among the bisphosphonate investigators, I think it should be the alternative common name or needs elucidation.

3 In the abstract, page 2, (in vitro or/and in vitro) should be (in vitro or/and in vivo)?

4 page 4, (classifid) should be classified.

5 The elucidation, YM529 (1-Hydroxy-2-imidazo….) should not be duplicated (page 4, 5) Dot (.) is not necessary before Equal (page 8).

6 In the Western blotting analysis using Ras antibody, there should be two bands which indicate unprenylated and prenylated forms of Ras. The prenylated and unprenylated forms of Ras move differently in the gel electrophoresis. Moreover, the activated and prenylated forms of Ras should be in the membranous fractions, whereas inactivated and unprenylated forms of Ras should be in the cytoplasmic fraction. I think authors can show the growth inhibition of NSCLC cells by YM529 would be due to Ras dependent pathway or not. I could understand author speculated cellular apoptosis was induced thorough the reduction of the phosphorylation of the ERK1/2 because phosphoERK1/2 was reduced after YM529 treatment. However, authors did not show that YM529 inhibited the prenylation of Ras. I think the authors should investigate whether the alteration of ERK1/2 signal is Ras-dependent or Ras-independent pathway. Separation of membrane and cytoplasmic fractions is one way to show it.

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**Discretionary Revisions (which the author can choose to ignore)**
**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

I declare that I have no competing interests.