**Reviewer's report**

**Title:** Combination therapy with alfacalcidol and risedronate improves the mechanical property by ameliorating the biological apatite c-axis orientation of bone in ovariectomized rat model of osteoporosis.

**Version:** 1  **Date:** 21 November 2008

**Reviewer:** James K Yeh

**Reviewer's report:**

The experimental design was made primarily using 2-way ANOVA to evaluate the interaction of ALF and RIS treatment at “sub-therapeutic doses” in mid-aged 12-week OVX rats for another 12 weeks and authors concludes that combined ALF and RIS therapy at their sub-therapeutic doses can additively improve bone quality better than each therapy alone. The conclusion is based on the results of serum and urinary biochemical parameters, bone strength of lumbar and cortical femur, micro-architectural indices of lumbar vertebra, BMD, BAp c-axis orientation of the lumbar vertebra.

**Comments:**

Additive treatment effects is defined by the authors as”the sum of the interaction between the two treatments, were assessed using a significance level of P=0.10. If the interaction was not statistically significant (P>0.10), the effects of ALF and RIS were considered to be independent of each other, and the effects of ALF and RIS were considered additive when there were given in combination”. It is problematic of the definition. First of all additive effect is the absence of interaction (independent of each other, not the sum of the interaction), but a sum effect of the two treatments. Secondly, why use p value at 0.10 instead of 0.05? Since the title of the paper and the major issue of the study are evaluating the additive improvement of the combined treatment in each parameter measured, it is essential to show the result with 2-way ANOVA for each important parameter and demonstrate no interaction but a sum improvement effect of the two agents.

Table 2: ALF and RIS interaction should be ALF X RIS (not ALF+RIS). It should also examine any sum improvement effect to conclude an additive effect or not. Table 2 should include the actual values, one-way ANOVA significance, 2-way ANOVA ALF factor, RIS factor, and ALF-L X RIS-L interaction significances, from the OVX, ALF-L, RIS-L, ALF-L+RIS-L groups for several major parameters, such as L5 strength, femur strength, uCT indices, BMD and BAp c-axis orientation data, et al in order to show if there is any improvement by the combine intervention and in Discussion.

Example: It is obvious to see the additive effect on bone strength at lumbar vertebra (Figure 2A); ALF-L vs OVX (the actual values is higher than OVX, but NS), RIS-L is about the same as OVX, however, ALF+RIS is significantly higher
than the OVX, thus, better than either alone and improved and Interaction is NS either.

However, Bone density at Figure 4 A, the result of ALF+RIS is about the same as the ALF-L alone. Thus, with or without RIS, there seems no any improvement in the bone mineral density measured. It has to be precaution in the Discussion.

Figure 3: It is fine to use figures to illustrate the additive effect, but it is not necessary unless list most of the important parameters instead choose two to cover all.

Experimental Design: The rats were divided into 7 groups based on the BMD of lumbar vertebrae (L2-L4). The method of in vivo scanning should be described in the Methods.

Second paragraph of the Discussion: "These results agreed with our previous findings in demonstrating that ALF improved bone dynamics by a unique stimulating bone formation (6, 24)"

The current finding measured only serum OC as bone formation marker and the result do not show any change.
You may discuss and compare the dose used in the current study with previous reports.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**
I declare that I have no competing interests.