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: 5 December 2008

Reviewer: Matthew Allen

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Major Compulsory Revisions

1. Please strongly consider revising the title of the study. Although combination treatment with ALF and RIS improved mechanical properties and changed the c-axis orientation in bone, there is no evidence presented showing 'causation'. To say that one occurred because of the other is not reflective of the information presented in the paper.

2. The introduction paragraph on BAP c-axis is confusing as it fails to define what Bap c-axis orientation is and does not sufficiently explain how this parameter changes under various conditions (e.g. is it altered by remodeling, age, etc) or how it alters mechanical properties. This information is essential as this is not a traditionally assessed parameter thus many readers will not be familiar with such a measure.

3. The final sentence of the introduction necessitates clarification. Statements such as 'focused on differences in the mechanisms whereby RIS and ALN act on bone dynamics' are vague and unclear (the second sentence of this paragraph is much better). Please state explicitly what the goal of the study was and also please state the hypothesis. Please insert the word 'changes' in the final sentence such that it reads 'ameliorating changes in mechanical properties'.

4. It is not clear why only ultimate load was reported? At a minimum, the other extrinsic properties (stiffness, energy absorption) should be assessed from the load-deformation data and included. Ideally, some of the intrinsic material properties (ultimate stress, modulus, toughness) would be presented also. Ultimate load is only part of the story with respect to fractures so in the quest to optimize treatment for fracture risk reduction it is necessary to examine the a more complete compendium of biomechanical properties. For example, it is possible to have a bone that is very strong (high ultimate load) but fractures in a brittle fashion (for example osteopetrotic bone). So if one only considers strength, it is possible to miss important information. At an absolute minimum, this needs to be addressed in the discussion.

5. The use of 0.10 as a significance value is certainly not traditional. Please either provide strong justification for doing so, or use a more traditional value of 0.05.

6. The results/discussion repeatedly refers to dose-dependent changes. While in
most of these cases, the two doses of either ALF or RIS appear different, in few of the cases do the data show the two doses are significantly different from each other. Without significant differences between the two doses of a compound, it is not valid to say there is a dose-dependent effect.

7. The first paragraph of the discussion necessitates major revision. There is no data presented to show that the amelioration of Bap c-axis was responsible for the differences in mechanical properties (as implied). Also, to say that microarchitectural structure was maintained when only trabecular spacing (not trabecular volume, thickness, or number) was changed in the combination group is a bit of an overstatement. Terms such as ‘destroyed’ should be removed. The final sentence is too vague as the combination therapy was only better than monotherapy for ultimate strength in some cases (e.g. in the LV the combination was better than RIS but not ALF; in the femur the combination was better than ALF but not RIS). One cannot make such broad statements without a broad effect.

Minor Essential Revision

1. The paper would benefit if it was edited for proper English as there are several areas that are missing words.

2. The introduction suggests bisphosphonates reduce fractures by increasing mineralization rather than maintaining bone mass/architecture. This is not a majority view and the evidence far more favors the preservation of mass/architecture although there clearly is some role for mineralization. Please consider revising this text.

3. Please state the total number of animals, as well as the number in each group.

4. Please provide additional information on dosing. For example, the dose of RIS (0.3 mg/kg) is about 3x higher than what is given to post-menopausal women (5mg/day to 60 kg women = 0.8mg/kg). It would be useful to explain how the doses were chosen, with references where appropriate.

5. Can the authors please clarify where the BMD measures were made (cortical or trabecular bone)?

6. What does the statement that Bap c-axis is a useful parameter for evaluating ‘history of bone formation’ mean?

7. The methods concerning Bap assessment are unclear. It seems as if measures were made on a cross-section in the ventral cortical bone, yet later it says the bones were cut and polished to the center? Please clarify.

8. Please only report the data as SE or SD.

9. Please explain in more detail the way additive effects were assessed. What does the ‘sum of the interactions between two treatments’ mean? It is also unclear what is meant by the effects of ALF and RIS were considered additive when given in combination if the interaction was not significant?

10. The text on interactions suggests that the lines on figure 3 need to intersect for there to be an interaction. This is not true and thus the text (and possibly the
method for assessing interaction) should be revised.

11. The value of Figure 5 is unclear. Please either emphasize why this is important or remove. The text regarding the ‘optimal state of bone material parameters’ is not supported by the data.

12. The statement about ‘restoration of damaged trabeculae’ is not supported by the data.

13. The text on a ‘unique mechanism’ with respect to changes in bone formation and resorption should be removed as the biomarker data are not sufficient to make such a claim. Furthermore, the data that are cited as evidence for this uncoupling of bone formation and resorption do not unambiguously show it to occur.

14. The discussion suggests a goal of the study was to find ways to minimize adverse reactions to treatment but it’s not clear how this study really addresses such a question.

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

**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 have received research funding from Amgen, Eli Lilly, and Procter & Gamble and served as a consultant for Merck and Procter & Gamble.