Author's response to reviews

Title: Changes in serum vitamin D and PTH values using denosumab with or without bisphosphonate pre-treatment in osteoporotic patients: A short-term study

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Author's response to reviews: see over
Dear Editor,

We would like to re-submit our manuscript, entitled “Changes in serum vitamin D and PTH values using denosumab with or without bisphosphonate pre-treatment in osteoporotic patients: A short-term study”, for publication in BMC Endocrine Disorders.

Please find below our rebuttal letter containing point-by-point responses to the Reviewers’ comments. In addition, the manuscript was re-read by a native English-speaking scientist.

We hope that the revised version is now suitable for publication in BMC Endocrine disorders. Thank you for your time and we are looking forward to your reply.

Sincerely,

Yukio Nakamura

Reviewer's report:
Nakamura and colleagues report a small study looking at the impact of pretreatment with bisphosphonates prior to denosumab on markers of bone turnover and related variables. They show a difference between the two groups and propose differences in early mechanisms of action of denosumab between the two groups. Accepting that the study was very small, some of these observations are of potential interest.

Major
1. The study is very small with only 11 in each arm and thus it is difficult to draw any firm conclusions from such a small sample size.

→Thank you highlighting this point. After considerable revision, we have emphasized our major findings to be that denosumab imparted stronger bone absorptive effects after BP therapy and that bone formation markers decreased thereafter. Although this study indeed contained a limited number of samples, bone absorptive markers were significantly decreased after 1 week of denosumab administration. Thus, we believe that our conclusions may still be
drawn despite this acknowledged limitation.

2. The authors state that hypocalcaemia is a frequent complication of denosumab. Clinically relevant hypocalcaemia is not a frequent complication and thus this needs some clarification within the text. When hypocalcaemia is seen, it is often in the setting of untreated vitamin D deficiency and/or significant chronic renal impairment.  
  → We deleted this part in the Introduction section based on the Reviewer’s comment. Vitamin D was given to all patients in this study.

3. Do the authors believe that the observed differences in serum calcium following denosumab injection have any clinical relevance? The changes in adjusted calcium are clearly within the reference range.  
  → We appreciate this question. We think that there was no clinical relevance with respect to serum calcium following denosumab injection and have stated that the changes in adjusted calcium were clearly within the reference range in the Results section. Since we could not reach a clear conclusion on serum corrected calcium levels due to the small size of the cohort, we have added this point as another limitation.

4. What was the pretreatment management of vitamin D status in these patients?  
  → Vitamin D was administered to all patients in the study. We have presented the data on 1,25OHD₃ before treatment, but did not examine 25OHD level in each patient. We are currently preparing another manuscript showing no observable changes in 25OHD level after long-term BP treatment.

5. It is stated in the text that denosumab has some bone forming action. This is rather an unconventional proposal and needs further expansion if the authors stand by this conclusion. I assume the authors mean that bone formation was inhibited less than bone resorption but this needs some clarification.  
  → Thank you very much for this helpful comment. We have edited the text as follows: “…following pre-BP treatment, the inhibitory effects on bone formation markers by denosumab were much less pronounced than those in the denosumab alone group.”
6. Text is 25% too long.
   → We have deleted roughly a quarter of the text according to the Reviewer’s advice, most notably in the Introduction and Discussion sections.

Minor
1. Most acceptable terminology for serum calcium is adjusted for albumin.
   → Thank you for pointing this out. As all serum calcium levels were adjusted for albumin, we have described this in the text.

2 Minor adjustments for use of English needs checking throughout.
   → A native English-speaking scientist has revised the paper.

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
   → As an expert on statistics, our co-author Dr. Ikegami has evaluated the differences contained in the study. Although the main limitation was a small sample size, statistically significant differences were found for values at various time points using linear mixed models and Holm’s correction method for multiple comparisons. The timing of the measurements was used as a fixed effect while the individuality of the measurements was adopted as a random effect. We also addressed this issue with another statistical expert, Professor Akira Taguchi, who confirmed the validity of our statistical analyses. He has been added as a co-author.

Reviewer’s report:
This is a small study with findings of interest. However, the findings are considerably overstated and lots read in to small differences or lack of differences most likely due to small sample sizes. Much of the data relating to changes in active vitamin D and PTH and the complicated explanations are difficult to believe given that the patients were treated with calcium and vitamin D as well as the DMab.
Major:
1. Last line of abstract does not read well. What does this mean?
   → We apologize for our lack of clarity and have re-phrased the last sentence.
2. I think it is wrong to say that denosumab has bone forming properties in the abstract conclusion as this is only in a restricted group of patients at one time point.
   
   →Thank you very much for pointing this out. We have edited the text as follows: “…following pre-BP treatment, the inhibitory effects on bone formation markers by denosumab were much less pronounced than those in the denosumab alone group.”

3. Need to say what the type of BP treatment was. Oral or IV.
   
   →We have clarified in the Methods section that oral BPs were administered.

4. Need to say whether any individual had a serum calcium outside the normal range (high or low).
   
   →We have more clearly stated that the changes in adjusted calcium were clearly within the reference range in the Results section. No patients had a serum calcium level outside of normal range. Since we could not reach a clear conclusion on serum corrected calcium levels due to the small size of the cohort, we have added this point as another limitation.

5. The whole issue of bone formation markers was not covered well. DMab will decrease formation in the long term.
   
   →We agree with the Reviewer. As stated in the text, we observed that the inhibitory effects on bone formation markers by denosumab treatment were much less pronounced than those of pre-BP treatment. Since this is a short-term study, we are preparing to report on a long-term follow-up cohort in the near future.

6. Second line of the discussion does not seem appropriate. These drugs have not been compared directly and inferences regarding their anti-fracture effects cannot be drawn.
   
   →Thank you for pointing this out. We have deleted this part of the text.

7. Again, the discussion about coupling is only based on a small number of patients and this data is not solid compared to the data based on thousands of patients in the RCTs of these drugs. This study did not have a control arm or a placebo arm so many of the conclusions may not be as the authors suggest.
Thank you highlighting this point. After considerable revision, we have emphasized our major findings to be that denosumab imparted stronger bone absorptive effects after BP therapy and that bone formation markers decreased thereafter. Although this study indeed contained a limited number of samples, bone absorptive markers were significantly decreased after 1 week of denosumab administration. Thus, we believe that our conclusions may still be drawn despite this acknowledged limitation.

8. There was only 1 line regarding limitations. Were these really the only limitations?

Since we could not reach a clear conclusion on serum corrected calcium levels due to the small size of the cohort, we have added this point as another limitation. However, apart from these changes in calcium values, we feel our significant results will be of interest to journal readers interested in denosumab therapy.

9. A medical services writer was used. However, many parts of the manuscript were difficult to understand. It was unclear if this was a problem with the authors not being clear in their own minds or whether this was a translation problem.

After modifying the paper based on the Reviewers’ comments, a native English-speaking scientist has re-revised the text.

10. Looking at figure 2, denosumab leads to a similar reduction in both bone formation markers to the baseline in the BP treated group. There is thus no evidence that denosumab has less impact on bone formation than BPs.

Thank you for raising this point. We have edited the text to state that the inhibitory effects on bone formation markers by denosumab treatment were much less pronounced than those caused by pre-BP treatment.

Furthermore in people who have previously had BP the level of bone formation marker appears to decrease even further at the last time point (even if the low numbers mean this is not significant). Thus DMab appears to have a more powerful effect that BPs to suppress bone formation.

We did not compare the bone inhibitory effects with the values of bone formation markers in patients treated with BP or DMab only. Thus, we could not draw the conclusion that DMab may have had a more powerful effect than BPs.
to suppress bone formation.

11. It would be helpful to have a clear statement that the authors have no received hospitality, honoraria, travel expenses etc via the companies that market denosumab.
→We have added this statement into the text.

**Statistical review:** No, the manuscript does not need to be seen by a statistician.
→As an expert on statistics, our co-author Dr. Ikegami has evaluated the differences contained in the study. Although the main limitation was a small sample size, statistically significant differences were found for values at various time points using linear mixed models and Holm's correction method for multiple comparisons. The timing of the measurements was used as a fixed effect while the individuality of the measurements was adopted as a random effect. We also addressed this issue with another statistical expert, Professor Akira Taguchi, who confirmed the validity of our statistical analyses. He has been added as a co-author.